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Manatū Ahu Matua

Source attribution January to December 2015 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 of human *Campylobacter jejuni* cases from the Manawatu, 2015.

Source attribution is the process of determining the proportions that various pathways and sources contribute to the prevalence 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 based on 2015 data. It describes the results of multilocus sequence typing and source attribution modelling of isolates lodged in the MPI funded culture bank of *Campylobacter jejuni* samples from animals and humans in the Manawatu sentinel site. These were catalogued and stored at mEpiLab, Massey University.

The 165 primary human samples yielded 122 full MLST allelic profiles. Reservoir attribution modelling revealed that 45% - 70% (credible intervals) of the human cases could be attributed to poultry and 25% - 50% to ruminants. Sequence type ST-6964, which was first discovered in New Zealand in 2014, was the dominant poultry type (31% of isolates) and was identified from four human cases but no other non-poultry sources. Of the 128 pooled faecal ruminant samples, 65 were positive for *C. jejuni* and 11 for *C. coli*. STs not previously seen were identified.

The marked decrease in human cases attributable to poultry reservoirs, subsequent to the introduction of the *Campylobacter* in Poultry Risk Management Strategy in 2006, is still evident in 2015. In the pre-intervention period over 70% of human cases were attributable to poultry, whereas in the post intervention years this estimate has declined to around 50%. Despite a proportional increase in the contribution of ruminants to human cases (25 to 37%), poultry strains still remain the most important source of human infection in the Manawatu. There is a clear difference in the attribution of the rural and urban cases, with ruminants being more important for rural dwellers and poultry for urban ones.

It is important to note that these ongoing studies only evaluate the animal reservoirs associated with strains and do not examine the pathway of exposure to *Campylobacter*, e.g. direct animal contact, meat from the reservoir animal, other foods, drinking or recreational water, etc.

Further studies at a national level to examine risk factors and source attribution encompassing epidemiological and genomic approaches are being explored by MPI.

MASSEY UNIVERSITY
COLLEGE OF SCIENCES
TE WĀHANGA PŪTAIAO

Final Report: MPI Agreements 17433 and 17509 Source attribution January to December 2015 of human *Campylobacter jejuni* cases from the Manawatu

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

June 2016

prepared for
the Ministry for Primary Industries

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mEpiLab

IDReC | Infectious Disease Research Centre

Report: MPI Agreements 17433 and 17509

Source attribution January - December 2015 of human *Campylobacter jejuni* cases from the Manawatu.

June 2016

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* and *Campylobacter coli* samples from poultry, ruminant, 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 2015. A total of 176 samples were submitted to the mEpiLab in this period, of which 165 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 165 primary samples, 129 were successfully cultured and 122 samples were sequence typed (2 were typed directly from the swab without culturing), yielding 122 MLST allelic profiles. Of these, 102 were *C. jejuni* that could be linked to EpiSurv data from 102 cases, the remainder were either *C. coli* (N=9) or could not be linked to EpiSurv data (N=11).

The new sequence type ST-6964 observed on poultry was the dominant strain in 2015, accounting for 31% of all poultry isolates. It was observed on carcases from poultry suppliers A, B, and other (supplier C, but not supplier D), and was also observed in 4 human cases throughout the year.

Reservoir attribution modelling revealed that 45–70% of human cases could be attributed to poultry with 25–50% attributed to ruminants in calendar year 2015. Given the uncertainty associated with these estimates, these are broadly similar to the trend since 2010, particularly as calendar year estimates have more variation year-on-year due to the summer peak in cases potentially shifting from one year to the next. When cases were divided into urban and rural dwellers, the attribution for 2015 showed that urban cases were more likely to be attributed to poultry (50–80%) while rural cases were more likely attributed to ruminants (30–80%). As in most years, there were relatively few (27) rural cases with MLST information, so there is significantly more uncertainty in rural attribution estimates. Dynamic attribution over time, accounting for the intervention in the poultry industry in 2007/2008, shows that rural attribution has been relatively unchanged, while there was

higher variation in the attribution of cases from urban dwellers, which is primarily due to variation in cases attributed to poultry.

A study of the rates of campylobacteriosis per DHB through time shows that DHBs that are predominantly urban experienced a large reduction in case rates following the intervention in the poultry industry on 2007/2008 where baseline case rates drop by around 50%. However, this is not seen as strongly in those DHBs that have a higher proportion of rural dwellers, where case rates in some DHBs have remained similar through time. This is consistent with rural dwellers being more likely to be in contact with animals and other environmental exposures.

MidCentral has a higher rural population (18.4%) compared to New Zealand as a whole (13.8%), and thus the reduction in case rates following the poultry industry intervention is not as clear in terms of average trend, predominantly being the result of a reduction in the summer peak which consists largely of urban cases. This is also highlighted by a change in seasonal trend due to a reduction in the magnitude of summer peaks. Analysing the urban and rural case rate trends separately shows the urban case rate dropping from approximately 80 cases per 100,000 population per annum to around 50 cases per 100,000 after 2007, while rural case rates remain relatively stable at similar (80 cases per 100,000 population per annum) rates as the urban cases 2005–2007. The change in seasonality in urban and rural cases is interesting, with the high summer peak noticeable before 2008 being replaced with a marked April/May dip and consequently more broad summer peak from 2010 onwards.

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 17433 between MPI and the Contractor for the period January 2015 to December 2015) 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 17433 between MPI and the Contractor) for multilocus sequence typing;
3. Collect cattle and sheep faecal samples from 32 Manawatu farms (as part of agreement number 17509) for multilocus sequence typing;
4. Use the sequence typing to populate dynamic source attribution models developed by the Contractor as part of agreement number 17433 between the Contractor and MPI; and
5. Prepare and submit a draft report to MPI for comment detailing the outcomes of clause 3.2 of the schedule; and
6. Prepare and submit a final report to MPI's satisfaction detailing the outcomes of clause 3.2 of the schedule.

3 Methods

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℃ 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℃ for 2 days before DNA preparations were made. Cultures were frozen at -80℃ in Glycerol Broth (Difco, USA).

3.1.2 Change in MedLab Central methods

The diagnosis of clinical campylobacteriosis can be performed routinely using ELISA, PCR or culture-based techniques. To date, the clinical diagnostic laboratory, Medlab Central, Palmerston North has relied on the ProSpecT® *Campylobacter* assay (ELISA) for campylobacterisosis identification in samples from patients exhibiting gastroenteritis. The efficacy of different detection methodologies was recently compared by mEpiLab researchers[2], and we present the key results below.

A total of 594 faecal samples from people with clinical gastroenteritis were tested by four diagnostic methods during 2014–2015. A PCR targeting five *Campylobacter* species (Lund PCR), 2 culture methods (CAT agar at 37℃ in a hydrogen-enriched microaerobic atmosphere and mCCDA at 42℃ in a microaerobic atmosphere) and the ProSpecT® *Campylobacter* assay (ELISA) were performed on each sample. From all samples, 109 (18%) tested positive for *Campylobacter spp.* by at least one method. Individually, Lund PCR detected 95 (16%), CAT 64 (11%), mCCDA 61 (10%) and ELISA 38 (6%) positive samples..

The ProSpecT® *Campylobacter* assay (ELISA) was the least sensitive of the tested methodologies. In addition, we know that the ELISA can also suffer from false positives, with some ELISA positives not being able to be cultured or grown in the Hopkirk laboratory. Just how the false positives and false negatives affect reported rates from MidCentral is unclear, though the comparison across DHBs presented in section 4.7 suggests they are not all that

far out of line with similarly rural DHBs.

Culture techniques proved more reliable and have comparable incurred costs to ELISA-based testing. As a result of these findings, as of 18 February 2016 Medlab Central, Palmerston North has changed its standard operating procedure for the diagnosis of campylobacteriosis to use the mCCDA culture-based methodology.

3.1.3 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℃, 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℃ microaerobically for 2 days. After incubation the broth was subcultured onto mCCDA agar and incubated microaerobically at 42℃ for 2 days. Single colonies resembling *Campylobacter* species were subcultured to BA and incubated microaerobically at 42℃ for 2 days before DNA preparations were made. Cultures were frozen at -80℃.

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℃ for 2 days. Colonies were counted manually or by using a plate reader (aCOLyte, Synbiosis, England).

3.1.4 Ruminant faecal samples

A total of 100 sheep, beef and dairy farms were selected at random from the Agribase™ 2015 database[1] (AsureQuality). While other researchers have

[1]The AgriBase™ database is established and maintained by AsureQuality.

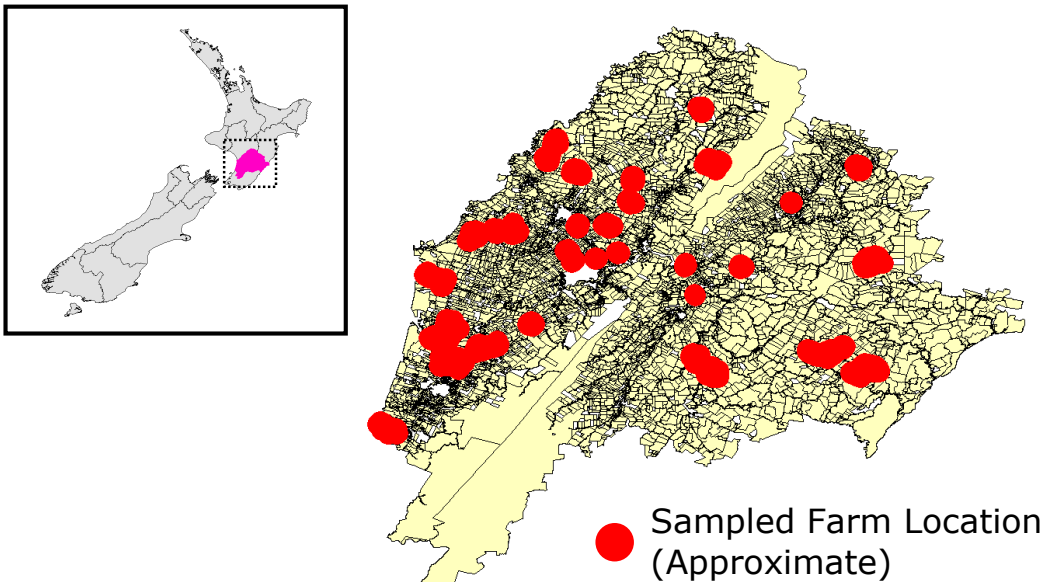

Figure 1: Approximate location of sampled ruminant farms.

found Agribase™ to be inconsistent with other similar information sources (FarmsOnLine, National Animal Identification) [6], and our experience suggests that it can be significantly out of date (with up to 25% being more than 3 years old) it is nonetheless a useful resource for finding potential farms to contact for sampling.

Farmers were contacted by letter and subsequently by telephone, and 32 farms (16 cattle and 16 sheep) from a broad geographical distribution were selected based on farmer approval for participation in the *Campylobacter* surveillance study. The approximate locations of the participating farms are illustrated in Figure 1. Of the selected farms, all 16 cattle farms are dairy farms, and 9 of the 16 sheep farms are sheep and beef, though only sheep faeces were sampled in this first sampling round.

Sampling from the 32 farms across the Manawatu district commenced in September 2015, with each farm being visited once in 2015 for collection, where 16 faecal samples were taken per farm. Samples were then pooled in sets of four and the pooled samples were screened for *Campylobacter*. Up to four isolates were obtained from each pooled sample using both selective Boltons/mCCDA and more permissive CAT-based enrichment protocols for speciation. If positive for either *C. jejuni* or *C. coli* the isolate was considered a candidate for sequencing, with at least one isolate of each species found

from each pooled sample being selected for sequencing.

3.1.5 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., [4]. Alleles that did not give clear results were re-amplified and sequenced using primers sets published by Miller et al., (2005)[7]. Sequence data were collated by Dr Phil Carter at ESR, and alleles assigned using the Campylobacter PubMLST database (http://pubmlst.org/campylobacter).

3.1.6 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 2015 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 [10]. 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[2] 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 STs to be speciated based on a concensus of all isolates submitted to PubMLST rather than relying only on NZ isolates.

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 2015 were calculated using the Asymmetric Island model as described elsewhere [11, 5, 9]. A model was then run for all the preceding 10 years with results being presented for 12 monthly periods from July through June to minimise year to year variation in attribution due to movement in time of the summer peaks which tend to be poultry associated. If more than one isolate was typed from a source sample, only unique STs were included in the analysis.

Pooling of samples and selecting only those isolates whose STs are unique from the pooled sample has the potential to bias the ST distribution observed,

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

however it is unclear exactly how much any such bias would contribute. In our view, any such bias due to pooling is likely to be insignificant in comparison to bias introduced by the microbiological process necessary to produce isolates for typing.

3.2.4 Dynamic source attribution modelling: Island model

The Island model [11] 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 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, or to include covariates for the human cases by modelling the F_k probabilities by case through time. Let

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

where

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

Here X_{kt} 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. The models used in this report are:

1. The simple model where $X_{kt} = 1$, thus modelling a constant mean attribution for each source, where the residuals ϵ_{kt} will soak up variation in time.

2. The intervention model where $X_{kt} = \mathsf{Intervention}_t$, where $\mathsf{Intervention}_t$ is a factor variable that allows attribution to differ before and after the intervention in the poultry industry (2007/2008). Again, residuals ϵ_{kt} soak up additional variation in time.

3. The urban/rural model where $X_{kt} = \mathsf{Intervention}_t + \mathsf{Urban}_k$, where Urban_k is an indicator variable allowing attribution to differ between 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 rest of the model is as in case 2.

3.2.5 Estimating sequence type effects

This year we include attribution estimates computed using the new `sourceR` package for R. This is an evolution of the modified Hald model [8], which has been extended to include separate estimation of attribution across independent locations (e.g. urban and rural dwellers, or different centers) and independent time periods. In addition, the statistical model has been redesigned to allow type effects, source effects and uncertainty in the prevalence of each type on the sources to be estimated simultaneously.

The model is as follows. Let Y_{itl} be the number of human cases of type i at time t in location l. Then we assume $\mathbf{Y}$ is distributed according to

$$Y_{itl} = q_i \sum_j a_{tlj} p_{ijt},$$

where q_i is the type effect for type i, a_{tlj} is the source effect at time t in location l for source j, and p_{ijt} is the prevalence of type i on source j in time t. Thus, we assume that type effects don't change through time (i.e. they are a property of the bacteria such as survivability, pathogenicity and virulence), while source effects may differ between locations and times, as can the prevalence of each genotype on each source.

Both the human cases $\mathbf{Y}$ and the positive samples on each source $\mathbf{X}$ are fit jointly in a single model, allowing the uncertainty present due to both human and source sampling to be incorporated in both the proportion of cases attributed to each source and the prevalence of each type on each source.

We assume that source effects are independent, while type effects follow a Dirichlet process, a non-parametric clustering mechanism that allows the effective number of distinct type effects to be chosen automatically, as we know that type effects are likely to cluster, with many having small effect due to not being observed among human cases.

Once fit, the posterior proportion of cases P_{jtl} attributed to each source j at a given time t and location l may be evaluated from each posterior sample of a, q, and p using

$$P_{jtl} = \frac{\sum_i q_i a_{tlj} p_{ijt}}{\sum_i \sum_j q_i a_{tlj} p_{ijt}},$$

which allows means and credible intervals to be computed.

3.2.6 Visualising genetic variation over time

In order to assess the genetic variation of *Campylobacter* over time, minimum spanning networks were generated from concatenated MLST gene sequences (aspA, glnA, gltA, glyA, pgm, tkt and uncA) for all genetically-typed New Zealand isolates from poultry, ruminants and humans that had been isolated

between 2005 and 2015. The PopArt software package[3] [1] was used for this, with the year of sample collection being defined as the sample trait to be mapped to each network node.

3.2.7 Statistical modelling of cases by District Health Board

As part of the epidemiological investigation for this report, we include analyses of monthly case rates from each District Health Board (DHB) in New Zealand from 2006–2015.

Numbers of cases per month by DHB were retrieved through the New Zealand Public Health Observatory (for cases from 2005–2014) and from the Monthly Public Health Surveillance reports[4] (ESR Ltd) for 2015 cases.

To compute rates, population counts per meshblock were obtained from Statistics New Zealand[5] from 2006 and 2013 census data releases and was interpolated to give population estimates per month per meshblock. Population per meshblock was then accumulated to give population estimates for each DHB per month.

The urban/rural status of each meshblock was derived from the 2013 and 2006 Meshblock datasets (Statistics New Zealand) allowing urban and rural populations to be estimated for each DHB, and thus allowing the percentage rural population to be estimated.

An R package `meshblocknz`[6] has been created to allow this information to be more readily available for use in this and future analyses.

Case rates per DHB (and for NZ as a whole) through time were modelled using seasonal decomposition of time series by local polynomial smoothing [3]. This method allows time series to be decomposed into seasonal, trend, and residual components, as follows:

1. The seasonal component is found by smoothing the seasonal sub-series (e.g. the series of all January values) using a local polynomial smoother.

[3] http://popart.otago.ac.nz
[4] https://surv.esr.cri.nz/surveillance/monthly_surveillance.php
[5] http://www.stats.govt.nz/Census/2013-census.aspx
[6] https://github.com/jmarshallnz/meshblocknz

2. The seasonal component is then subtracted from the time series, and the remainder is then smoothed, again with local polynomial smoothing, to estimate the trend.

3. The overall level (mean case rate) is then removed from the seasonal component and added to the trend.

The above process is iterated a small number of times to ensure trend and seasonal components are robustly estimated. We present smooth trends overlaid on actual case rates, and seasonal components with residuals overlaid in separate figures.

4 Results

4.1 Human samples

4.1.1 Human sample information

A total of 176 samples were submitted to the mEpiLab in this period, of which 165 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 165 primary samples, 129 were successfully cultured and 122 samples were sequence typed (2 were typed directly from the swab without culturing), yielding 122 full MLST allelic profiles. Of these, 102 were *C. jejuni* that could be linked to EpiSurv data from 102 cases, the remainder were either *C. coli* (N=9) or could not be linked to EpiSurv data (N=11)[7]. Note that the number of EpiSurv reports in Table 1 are obtained from the Monthly Notified Disease Surveillance Reports published by ESR[8], and that these differ from the number of EpiSurv data available to be linked to primary samples, primarily as the regions differ.

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.

[7] These can be for a number of reasons, most usually due to the patient not being able to be contacted for follow-up by public health officers, but may also be due to errors in labelling of samples, incorrect NHI numbers, or due to the sample being from a person located overseas.

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

2015					
Month	No. samples	Growth	%positive	EpiSurv reports	%coverage
January	12	9	75.0	19	63.2
February	16	11	68.8	14	114.3
March	19	10	52.6	26	73.1
April	2	2	100.0	5	40.0
May	3	3	100.0	3	100.0
June	8	5	62.5	9	88.9
July	8	8	100.0	12	66.7
August	10	9	90.0	10	100.0
September	23	20	87.0	25	92.0
October	10	8	80.0	12	83.3
November	31	27	87.1	33	93.9
December	23	17	73.9	28	82.1
Total	165	129	78.2	196	84.2

Table 1: Details of *Campylobacter* spp. ELISA positive human samples submitted by MedLab Central in 2015. 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.

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	17	81.0	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	19	13	68.4	20	95.0
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	203	158	77.8	262	77.5

Table 2: 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 3: 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.

4.1.2 Distribution of MLST genotypes of human cases

The proportion of human cases with each ST in 2015 is compared with previous years in Table 4. The previously dominant ST-474 accounted for just 4.9% of cases in 2015. This year the most common ST was ST-45 accounting for 18.9% of cases, with ST-42 and ST-61 accounting for 5.7% and 5.7% of cases respectively. These are all reasonably common strains. A handful of types not previously observed in humans were found this year in lower numbers (ST-486, ST-977, ST-2895, ST-3105, ST-4159, ST-4684, ST-5655). A future report will look at performing a rarefaction analyses of human, poultry and ruminant samples to assess how frequently we would expect to find new, previously unobserved strains, though with *Campylobacter* constantly evolving, it is likely that there will always be a small number of new strains discovered over time.

The genetic variation of *Campylobacter* isolates from humans was analysed for all samples between 2005 and 2015 (Figure 2). Sequence types that were commonly occurring across all years included ST-61, ST-474, ST-50, ST-53, ST-48, ST-583 and ST-45.

ST	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015
21	0.7	1.4	1.0	2.2	0.9	0.7	4.0		3.3	1.3	3.3
25		0.5					0.8	0.9		0.6	0.8
42	3.5	2.9	4.0	4.4	3.8	3.0	4.8	5.2	4.0	5.2	5.7
45	6.3	7.7	11.6	6.7	10.4	11.2	12.8	13.8	12.6	8.4	18.9
48	5.6	11.5	6.0	3.3	5.7	4.5	6.4	6.9	4.0	0.6	1.6
50	4.2	2.4	8.5	7.8	6.6	13.4	8.8	7.8	5.3	10.3	4.9
53	9.1	4.3	4.0	14.4	4.7	8.2	7.2	7.8	7.3	7.7	2.5
61	3.5	2.4	3.0	5.6	4.7	6.0	4.8	6.0	5.3	7.1	5.7
137		0.5			0.9	0.7		1.7	0.7		2.5
190	7.7	4.3	1.5	7.8	0.9	2.2	4.0	6.0	6.6	0.6	4.9
354	1.4	3.3	7.5	4.4					1.3	2.6	1.6
422			1.5	1.1		3.0	0.8	0.9	1.3	3.2	0.8
436	0.7	0.5	1.0	5.6	0.9	2.2	1.6	1.7	1.3	1.9	0.8
474	25.2	36.4	24.6	17.8	29.2	11.2	5.6	4.3	3.3	3.2	4.9
486											2.5
508								0.9		3.9	0.8
520	2.8	1.4	1.0		0.9	3.0	8.8	2.6	1.3	3.9	2.5
583	0.7	1.0	3.5		5.7	3.7	2.4	0.9	5.3	1.3	4.1
677	0.7	0.5	2.0		0.9	1.5		0.9	3.3	1.9	2.5
854									0.7		0.8
977											0.8
1581	1.4	1.0		2.2		0.7		1.7	1.3		1.6
2026	2.1	2.4	1.5	2.2	2.8	0.7	2.4	0.9	2.0	2.6	2.5
2345	0.7	1.4			0.9		0.8		4.0	5.8	1.6
2895											0.8
3072		0.5	1.0	1.1		0.7		0.9		0.6	2.5
3105											0.8
3232	0.7					0.7					0.8
3538			0.5		0.9	0.7	1.6	2.6	0.7		0.8
3610				1.1			1.6				0.8
3711			1.0		0.9	3.7	1.6	0.9	0.7	0.6	0.8
3798							0.8		0.7	1.3	0.8
4009										0.6	0.8
4159											0.8
4337					0.9			1.7	0.7		4.1
4684											0.8
5655											0.8
6964										1.9	3.3
NEW					1.9			2.6		0.6	2.5
No. samples	143	209	199	90	106	134	125	116	151	155	122

Table 4: The distribution of *C.jejuni* and *C.coli* multilocus sequence types in human cases in 2015 compared with the distribution of the same STs in human cases in the preceding years.

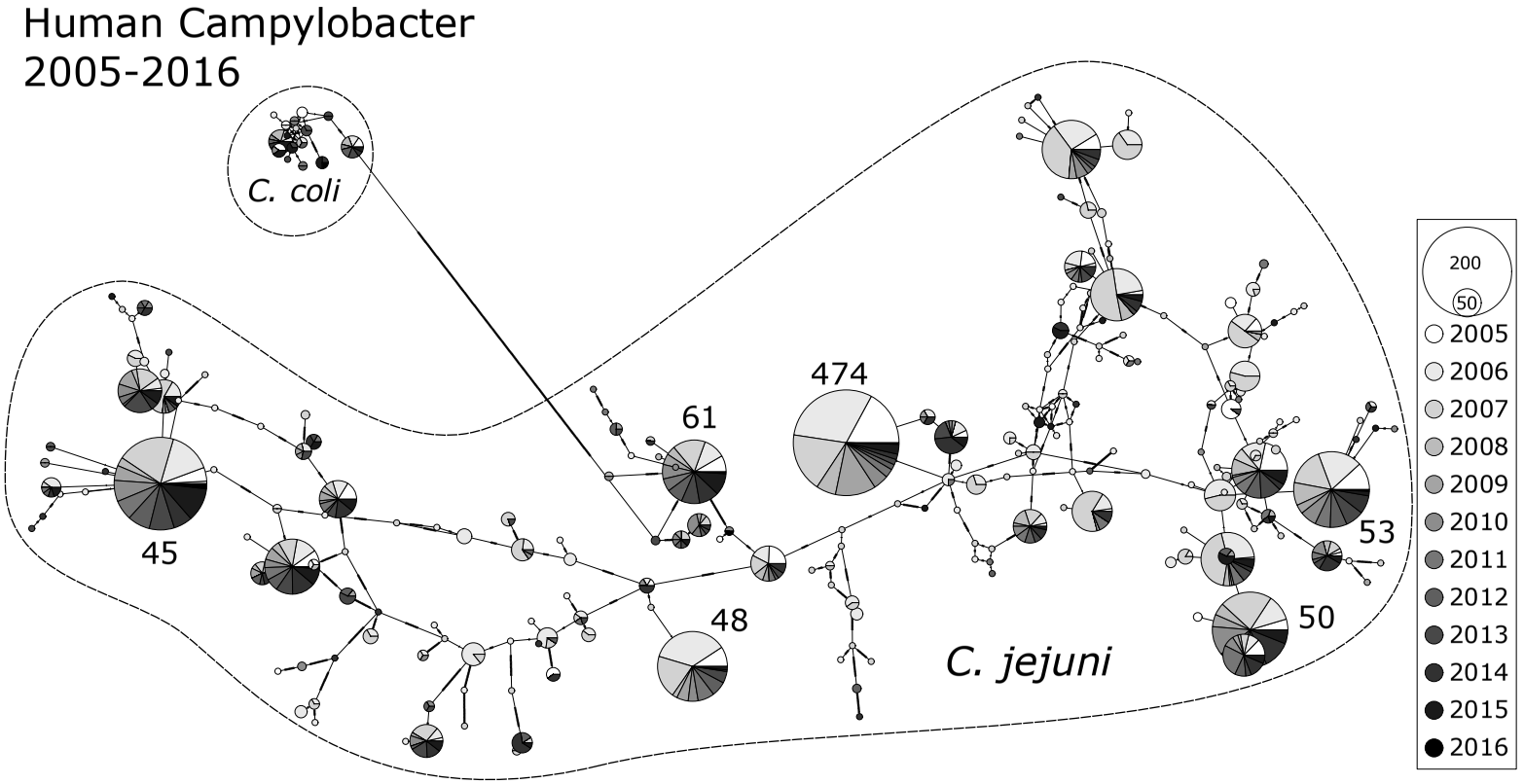

Figure 2: Minimum spanning network of New Zealand Campylobacter isolates from humans between 2005 and 2015. Node size denotes the number of genetically typed isolates belonging to each *Campylobacter* sequence type as specified in the key. Proportions of isolates from individual years of sampling are depicted using progressive shading. Key sequence types are labelled.

4.2 Poultry samples

4.2.1 Poultry sample information

As planned, 6 poultry samples were taken per month (N=72), of which 67 (93.1%) were *Campylobacter* positive. Of the 67, 76.1% (51) were confirmed as containing *C. jejuni*, and 19.4% (13) were confirmed as *C. coli*. Of these isolates, 7 were mixes containing both *C. coli* and *C. jejuni* STs. All suppliers yielded positive samples. The proportion of carcases positive for *Campylobacter* was 100% for supplier A, 86% for supplier B, and 93% for supplier Other. All suppliers had higher prevalence than the previous period (January–December 2014).

Company	Positive	Total	C.jejuni	C.coli	%positive	%2014
Supplier A	22	22	20	3	100.0	95.0
Supplier B	18	21	10	7	85.7	80.0
Supplier Other	27	29	21	3	93.1	81.5
Total	67	72	51	13	93.1	84.7

Table 5: The number of samples positive for presumptive *Campylobacter*, *C. jejuni*, and *C. coli* from each poultry supplier, and the percentage positive in 2015 compared to 2014.

4.2.2 MLST genotypes of poultry isolates

A total of 107 isolates from poultry were successfully MLST typed from 57 of the positive samples, with the most prevalent ST being ST-6964, the dominant ST across supplier A, B and Other, followed by ST-45 and ST-50 . Interestingly, there were 3 MLST profiles on supplier Other that were previously unobserved in NZ or in the PubMLST database.

The genetic variation of *Campylobacter* isolates from poultry was analysed for all samples between 2005 and 2015 (Figure 3). Sequence types that were commonly occurring across all years included ST-50, ST-48, ST-583 and ST-45. The epidemic of ST-6964 that occurred in poultry can be clearly identified within this data.

This year again sees quite a high prevalence of *C.coli*, a repeat of the pattern

	2013			**2014**			**2015**		
ST	A	B	Other	A	B	Other	A	B	Other
21									3.2
45	11.5	5.7	16.7		9.1	40.0	13.8		12.9
48		22.9			18.2				
50		8.6			13.6			15.8	19.4
51							3.4		
52							3.4		
53		2.9					6.9		3.2
190			8.3						
227			8.3						
257	7.7			4.5		4.0			
354	3.8			22.7		4.0			3.2
356				4.5					
520		2.9	25.0						
535	15.4			4.5					
538							6.9		
583	15.4	2.9		13.6		8.0	6.9		3.2
696							6.9		
825			8.3						
854		2.9	8.3					15.8	3.2
1581		17.1		9.1	18.2	4.0			
1590		14.3			9.1				
1900		5.7							
2256			8.3				10.3		6.5
2345	23.1			4.5			6.9		
2350		2.9							
2389									3.2
3072								5.3	
3105	3.8	2.9		36.4			3.4		
3230						12.0			
3721							3.4		
3792					4.5				
4009	7.7		8.3		9.1			21.1	
4337		5.7						5.3	
6964					18.2	16.0	27.6	36.8	32.3
NEW	11.5	2.9	8.3			12.0			9.7
No. samples	26	35	12	22	22	25	29	19	31

Table 6: The distribution of *C.jejuni* and *C.coli* multilocus sequence types in poultry in 2015 compared with the distribution of the same STs in poultry in the preceding two years (2013, 2014). No. samples refers to the total number of samples examined in each year.

seen in the last two years, with a number of types (ST-854, ST-2560, ST-4009) featuring (Table 7).

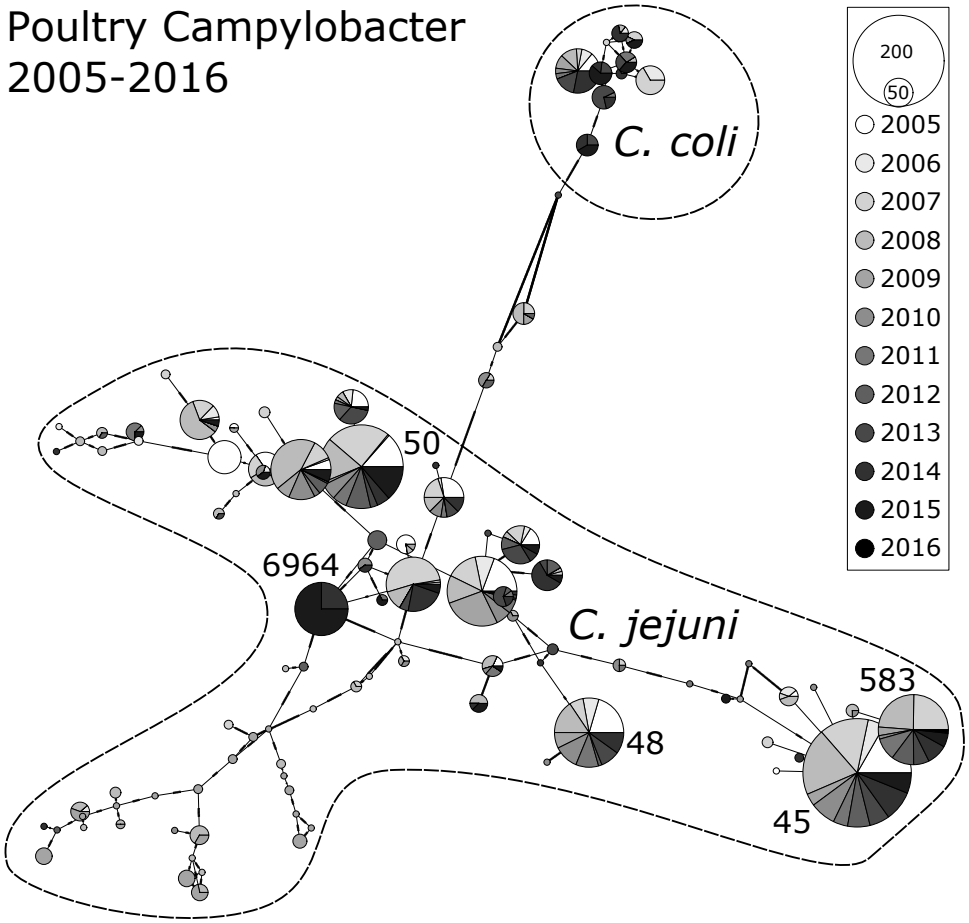

Figure 3: Minimum spanning network of New Zealand Campylobacter isolates from poultry between 2005 and 2015. Node size denotes the number of genetically typed isolates belonging to each *Campylobacter* sequence type as specified in the key. Proportions of isolates from individual years of sampling are depicted using progressive shading. Key sequence types are labelled.

The relatively new type ST-6964 has become the most abundant type found on poultry, across suppliers A, B, and Other (observed on supplier C but not supplier D). This sequence type was first observed in 2014, and was also found in 4 human cases in 2015. Table 8 lists the isolates by date and source.

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	93	43.1	6	2.8
2009	123	41	33.3	0	0.0
2010	72	41	56.9	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	14	19.4
2015	72	51	70.8	13	18.1
Total	1297	700	54.0	90	6.9

Table 7: The number and prevalance of *C. jejuni* and *C.coli* over the years 2005–2015. 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*.

Month	Human	Supplier A	Supplier B	Supplier Other
May 2014				2
Jun 2014				1
Jul 2014		1	1	
Aug 2014	2			1
Oct 2014			2	1
Nov 2014	1			1
Dec 2014			1	
Jan 2015			1	1
Feb 2015	1		1	
Mar 2015				2
Apr 2015				2
May 2015	1	2		
Jun 2015	1	1	2	
Jul 2015		1		1
Aug 2015		2	1	
Sep 2015	1		1	2
Oct 2015		2		
Nov 2015			1	1
Dec 2015				1

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 2015*.

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 4) and the estimated number of *Campylobacter* spp. given the carcase is positive (Figure 5). 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 Other 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 5), and has also been higher in the fourth quarter of 2015.

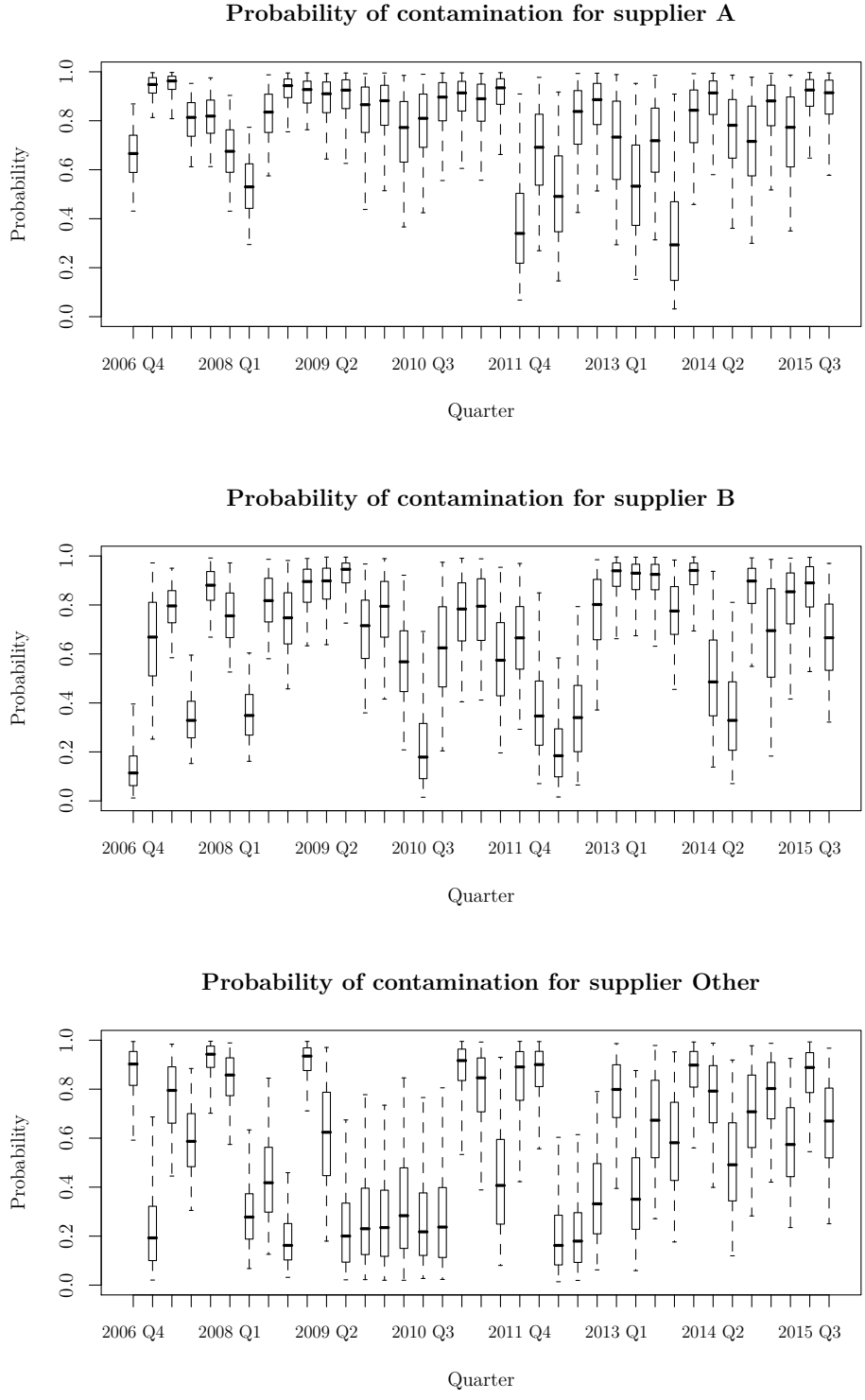

Figure 4: *Campylobacter* on chicken carcases by quarter: probability of contamination for each supplier, showing the median (thick horizontal line), interquartile range (box) and 95% (credible) intervals (dashed lines) of the posterior distribution.

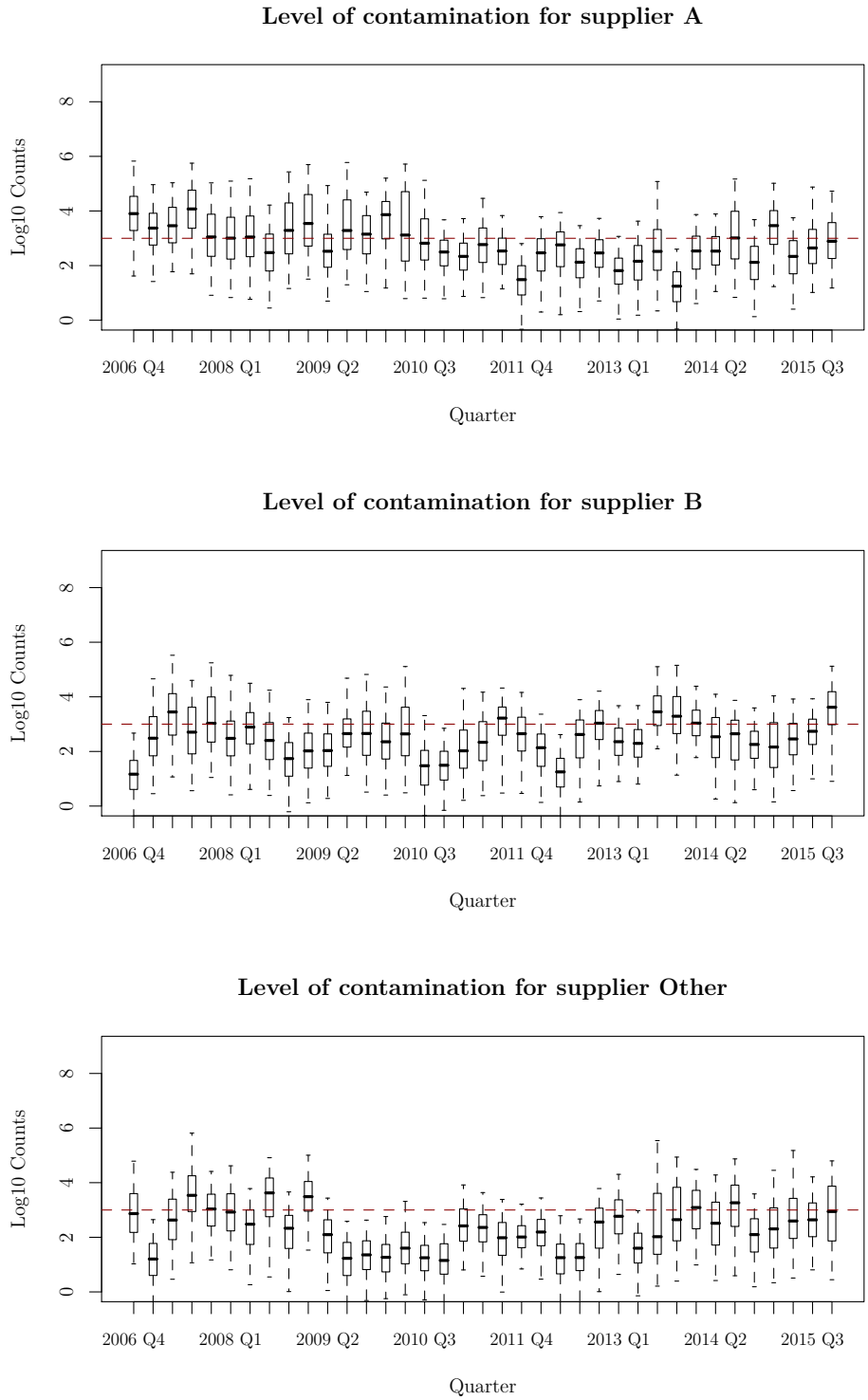

Figure 5: *Campylobacter* on chicken carcases by quarter: estimated level of contamination on positive carcases for each supplier, showing the median (thick horizontal line), interquartile range (box) and 95% (credible) intervals (dashed lines) of the posterior distribution. The dashed horizontal line marks 1000 cfu in the carcase rinsate.

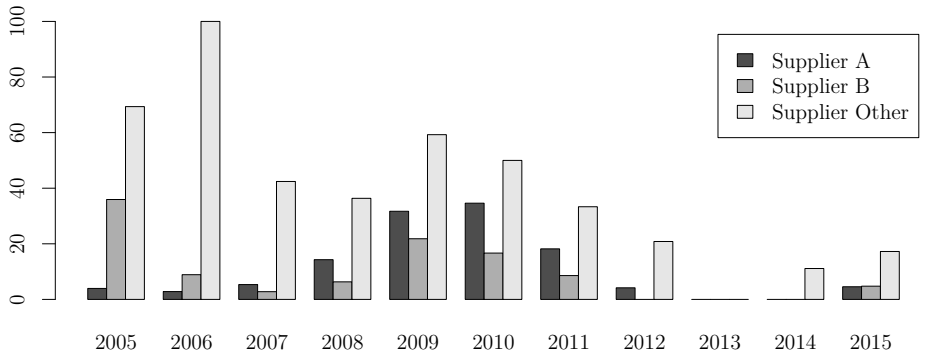

Figure 6: The percentage of chicken carcases with leaking packaging from each supplier from 2005–2015, N=1297.

4.2.4 Packaging of poultry carcases

The proportion of carcases from each poultry supplier with leaking wrappers was examined for the the whole 11 year period (1297 carcases). Differences between suppliers was observed, with supplier Other 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, though there has been a slight increase in 2015 (Figure 6).

4.3 Ruminant samples

4.3.1 Ruminant sample information

As planned, 512 ruminant samples were collected from 32 farms (16 samples per farm) across the Manawatu district between September 2015 and November 2015. Half of these (16 farms) were taken from cattle, and the other half were taken from sheep. Samples were pooled into sets of 4, yielding 128 pooled samples. Of these, 127 (99.2%) were positive for *Campylobacter*, with 16/16 cattle farms and 16/16 sheep farms having at least one positive sample. Of the 127, 51.2% (65), and 8.7% (11) were confirmed as *C. jejuni* and *C. coli* respectively. Of these isolates, 2 were mixes containing both *C. coli* and *C. jejuni* STs. Table 9 shows the number of positive samples by farm type.

Type	Positive	Total	C.jejuni	C.coli	%positive
Cattle	63	64	45	8	98.4
Sheep	64	64	20	3	100.0
Total	127	128	65	11	99.2

Table 9: The number of samples positive for presumptive *Campylobacter*, *C. jejuni*, and *C. coli* from each farm type, and the percentage positive from January to December 2015.

4.3.2 MLST genotypes of ruminant isolates

A total of 80 isolates from ruminants were successfully MLST typed from 74 of the 127 positive samples, with the most prevalent ST being ST-61 accounting for 12.5% of Cattle and 20.8% of Sheep isolates, followed by ST-50 and ST-42. A comparison of MLST types with types from 2005–2008 is given in Table 10.

The genetic variation of *Campylobacter* isolates from ruminants was analysed for all samples between 2005 and 2015 (Figure 7). Sequence types that were commonly occurring across all years included ST-53, ST-50, ST-42 and ST-61. All sequence types identified in previous rounds of sampling were also identified in 2015. Sequence types identified in 2015 that had not been identified in previous sampling rounds included *C. jejuni* types ST-257, ST-

403, ST-508, ST-767 and ST-6997 and *C. coli* types ST-1107 and ST-1581 as well as a number of isolates that are yet to have sequence type numbers assigned. Of these, sequence-types ST-257 and ST-1581 are known to infect other animals and humans. In addition, there were a number (4 in Cattle and 2 in Sheep) of types that as yet have no ST designation, indicating that they have not been submitted to PubMLST previously, accounting for 7.5% of isolates.

	2005-2008		**2015**			
ST	Cattle	Sheep	Cattle	Sheep	Poultry	Human
21	3.6	1.1	1.8		1.0	1.8
38	0.7		3.6		0.2	1.8
42	11.4	9.9	10.7		0.8	4.4
45	1.4	1.1	7.1	8.3	16.7	11.5
50	10.0	15.9	12.5	16.7	8.2	7.6
53	20.0	1.1		4.2	5.9	7.1
61	6.4	9.3	12.5	20.8	0.8	5.0
190	5.7	5.5		8.3	1.4	4.3
257			1.8		2.4	2.3
393		0.5		4.2		0.1
403			1.8			0.4
474	5.0	1.6	1.8		6.4	17.2
508			3.6			0.5
520	5.0	3.3	3.6		2.0	2.7
618	0.7	0.5		8.3		0.1
767			1.8			
1107			1.8			
1517		4.4	8.9		0.8	1.0
1581			12.5		3.8	0.9
2026	6.4	11.5		8.3		2.1
2392		1.1	1.8			
3072	2.9			8.3	0.3	0.7
3232		1.6		4.2		0.2
3711		1.1	1.8		0.2	1.0
3959	0.7		1.8			
6997			1.8			
NEW	0.7	0.5	7.1	8.3	1.8	0.6
No. samples	140	182	56	24	900	1456

Table 10: The distribution of *C.jejuni* and *C.coli* multilocus sequence types in ruminants in 2015 compared with the distribution of the same STs in ruminants in the 2005–2008. No. samples refers to the total number of samples examined in each period.

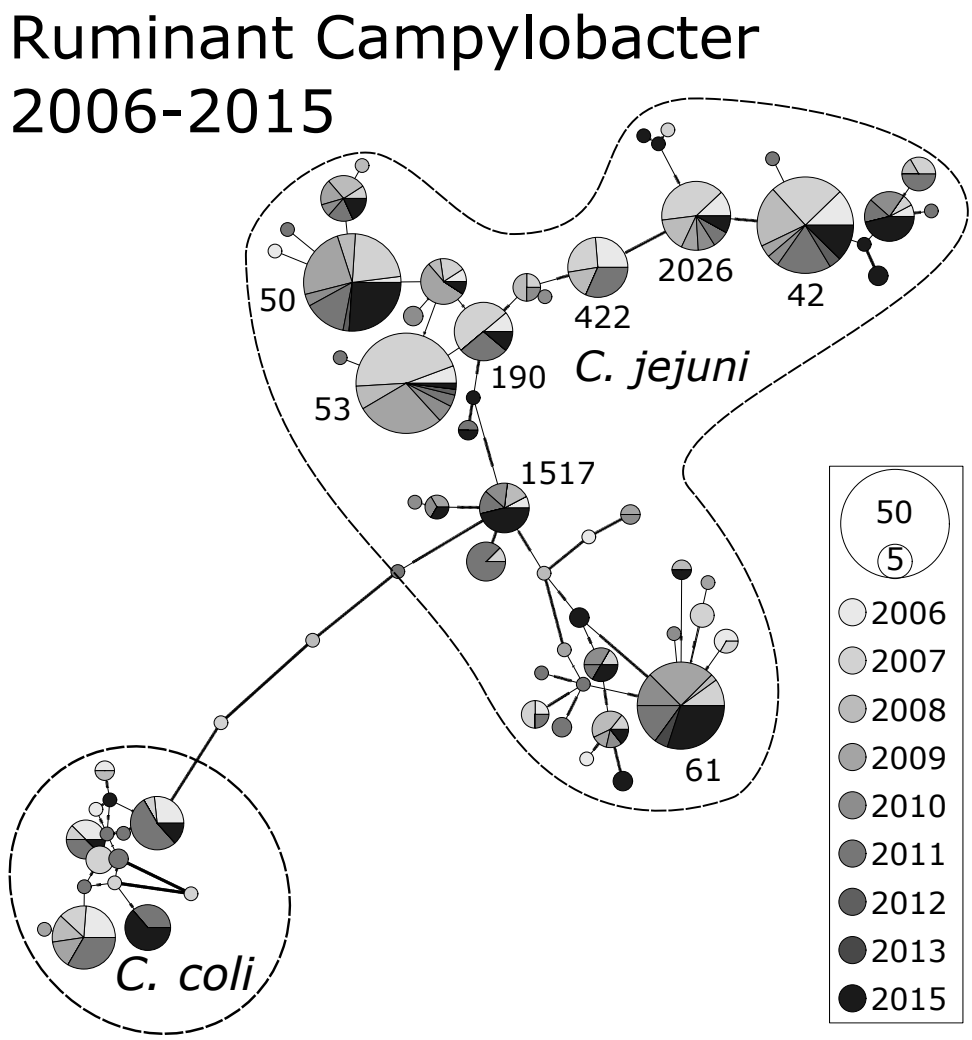

Figure 7: Minimum spanning network of New Zealand Campylobacter isolates from ruminants between 2005 and 2015. Node size denotes the number of genetically typed isolates belonging to each *Campylobacter* sequence type as specified in the key. Proportions of isolates from individual years of sampling are depicted using progressive shading. Key sequence types are labelled.

4.4 Samples available for attribution

There are a total of 3758 isolates with complete *C. jejuni* or *C. coli* allelic profiles available for source attribution, with an additional 76 isolates with partial profiles that could be uniquely assigned to a sequence type from PubMLST, giving 3834 total isolates available for attribution across the period 2005–2015. Source-specific totals are given in Table 11, and overall genetic variation of Campylobacter isolates from ruminants, poultry and humans is visualised for all samples in Figure 8. Note that ST-6964 appears

near many other poultry and human isolates. It is thus plausible that this isolate has either evolved from other similar strains in New Zealand, however it may have also been imported, as this strain has also been observed in China.

	Complete	Imputed	Total
Human	1543	12	1555
Supplier A	358	6	364
Supplier B	349	3	352
Supplier Other	252	3	255
Duck	49	1	50
Turkey	20	4	24
Spent Hen	26	1	27
Cattle	339	12	351
Sheep	267	11	278
Dog/Cat	33	1	34
Wild Water Bird	160	12	172
Other Wild Bird	81	3	84
Water	281	7	288
Total	3758	76	3834

Table 11: Number of complete and imputed *C. jejuni* isolates available for attribution for the years 2005–2015. Imputed isolates are those that have partial allelic profiles that match a unique sequence type in the PubMLST database.

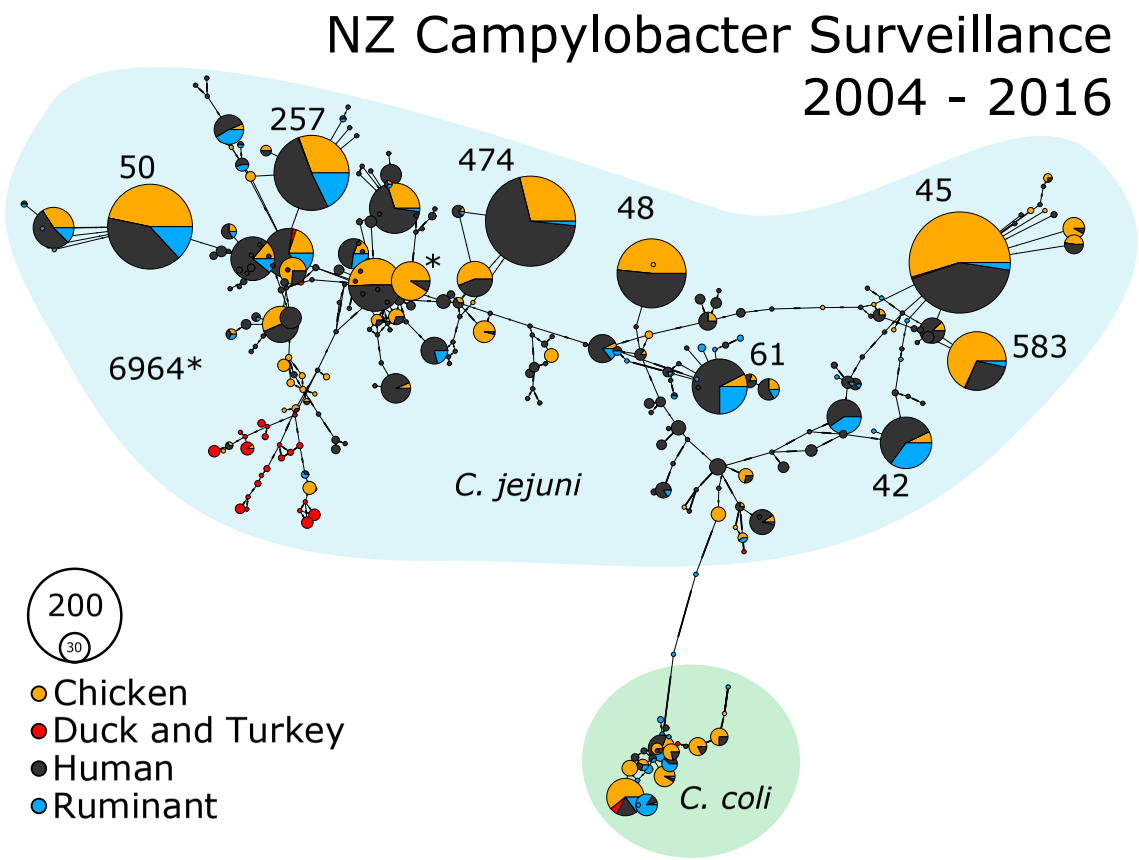

Figure 8: Minimum spanning network of New Zealand *Campylobacter* isolates from humans, ruminants and poultry between 2005 and 2015. Node size denotes the number of genetically typed isolates belonging to each *Campylobacter* sequence type as specified in in the key. Proportions of isolates from different source animals are indicated by the colours specified in the key. The most commonly identified sequence types are labelled.

4.5 Source attribution estimates for human cases in 2015 compared to previous years

4.5.1 Source attribution by year

Figure 9 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 2015. The proportion of cases attributed to poultry is up this year at around 50% with the majority of the rest being ruminant, particularly cattle sources.

Figure 10 summarises all sources over the 11 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 D), all ruminants (cattle and sheep) and other sources are presented in Figure 11. The complete table of estimates, including credible intervals is shown in Table 12.

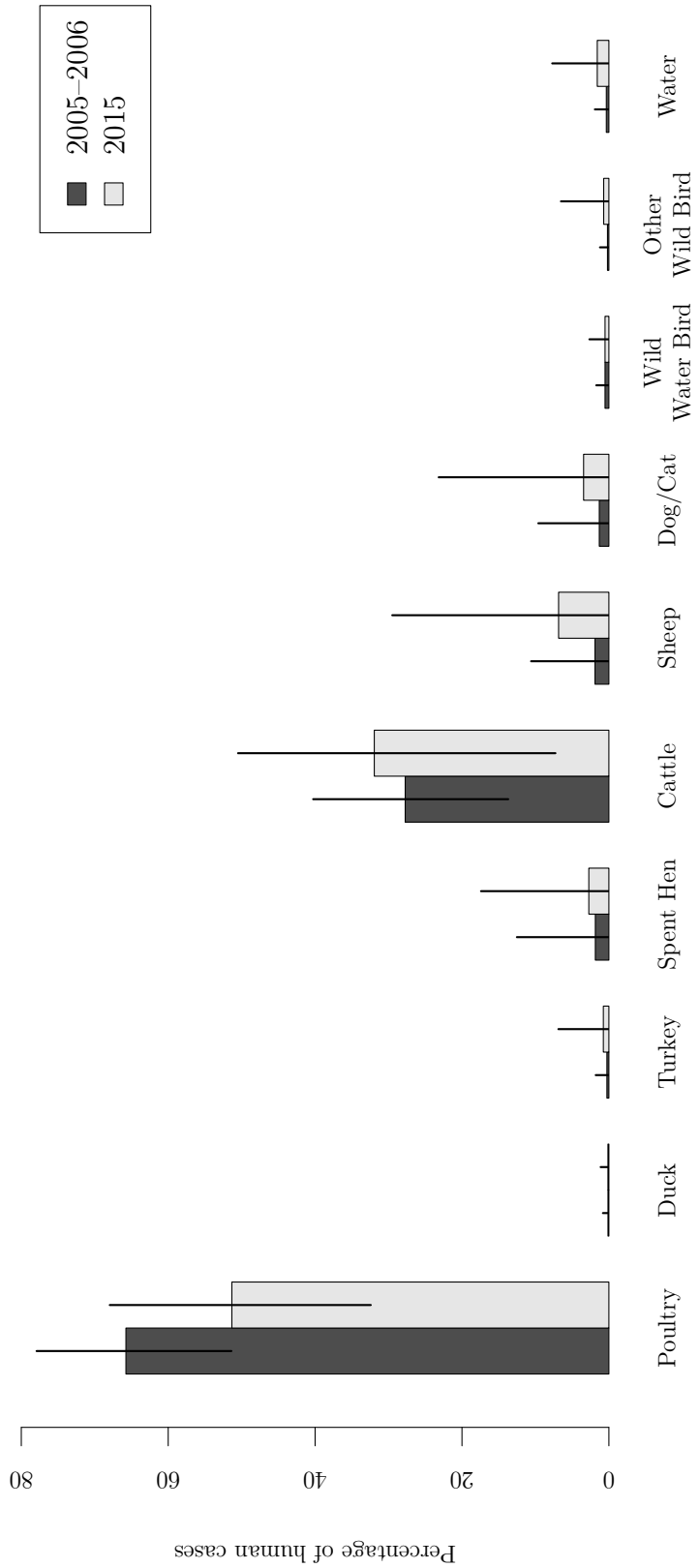

Figure 9: Source attribution for human cases in the Manawatu for cases reported between July 1st 2005 and June 30th 2006 compared to cases reported between January 1st and December 31st 2015. Error bars represent 95% confidence or credible intervals.

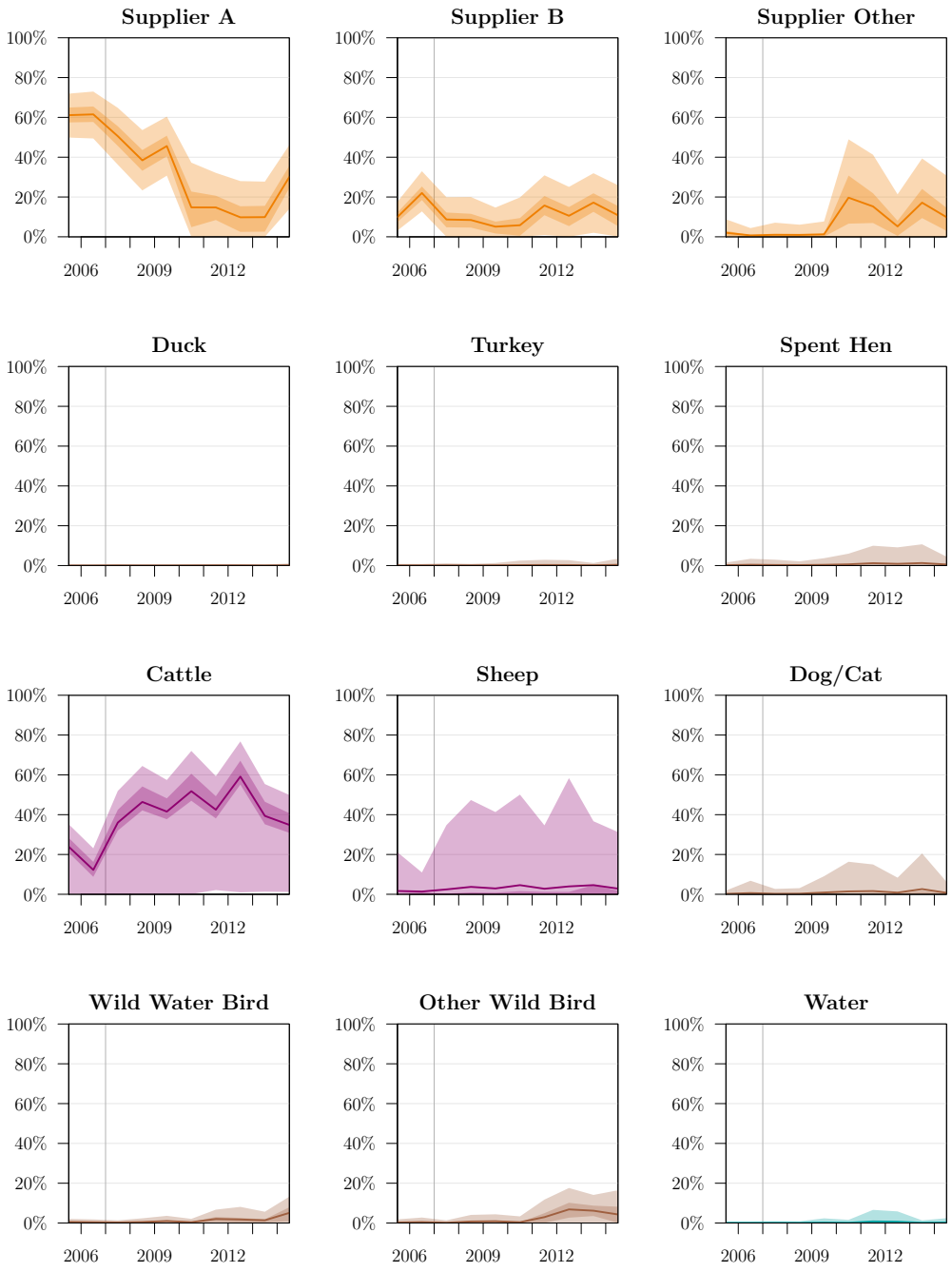

Figure 10: Poultry, ruminant and other source attribution estimates for human cases in the Manawatu between July 2005 and June 2015. Years 2006 and 2007 represent the pre-intervention period. Note that July to June is used to ensure the summer peak is centered within each period.

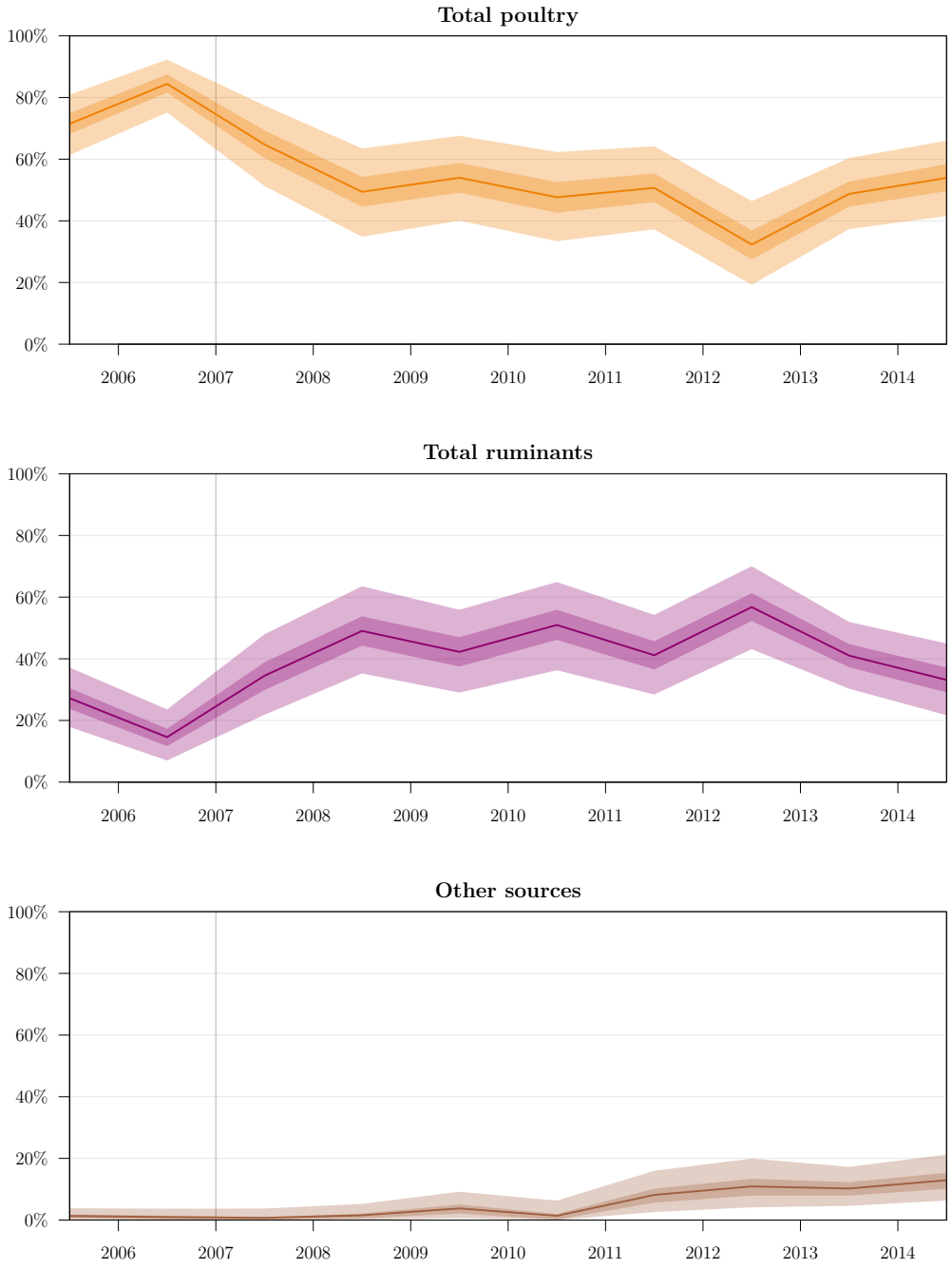

Figure 11: Poultry, ruminant and other source attribution estimates for human cases in the Manawatu between July 2005 and June 2015. Years 2006 and 2007 represent the pre-intervention period. Note that July to June is used to ensure the summer peak is centered within each period

	2005–2006	**2006–2007**	**2007–2008**	**2008–2009**	**2009–2010**	**2010–2011**
Supplier A	61.1 (49.9, 71.9)	61.5 (49.4, 73.0)	50.5 (36.2, 64.8)	38.4 (23.3, 53.6)	45.6 (30.7, 60.4)	14.8 (0.0, 37.3)
Supplier B	9.9 (3.1, 17.6)	22.0 (12.8, 33.0)	8.7 (0.0, 19.9)	8.4 (0.1, 20.0)	5.1 (0.0, 14.7)	5.8 (0.0, 19.8)
Supplier Other	2.0 (0.0, 8.8)	0.7 (0.0, 4.4)	1.0 (0.0, 7.1)	1.0 (0.0, 6.1)	1.3 (0.0, 7.7)	19.7 (0.0, 49.0)
Duck	0.0 (0.0, 0.4)	0.0 (0.0, 0.3)	0.1 (0.0, 0.6)	0.0 (0.0, 0.4)	0.1 (0.0, 0.5)	0.1 (0.0, 0.6)
Turkey	0.1 (0.0, 0.8)	0.1 (0.0, 0.8)	0.1 (0.0, 1.1)	0.1 (0.0, 0.8)	0.1 (0.0, 1.3)	0.2 (0.0, 2.5)
Spent Hen	0.2 (0.0, 1.5)	0.4 (0.0, 3.4)	0.3 (0.0, 3.0)	0.2 (0.0, 2.1)	0.4 (0.0, 3.7)	0.6 (0.0, 6.0)
Cattle	23.9 (0.2, 35.2)	12.3 (0.0, 23.1)	36.0 (0.1, 51.9)	46.4 (0.2, 64.5)	41.5 (0.2, 57.4)	51.8 (0.1, 72.0)
Sheep	1.7 (0.0, 21.1)	1.4 (0.0, 11.0)	2.4 (0.0, 34.7)	3.7 (0.0, 47.4)	2.9 (0.0, 41.3)	4.6 (0.0, 50.1)
Dog/Cat	0.2 (0.0, 1.9)	0.6 (0.0, 6.8)	0.3 (0.0, 2.7)	0.3 (0.0, 3.1)	0.9 (0.0, 9.1)	1.4 (0.0, 16.3)
Wild Water Bird	0.6 (0.0, 2.1)	0.4 (0.0, 1.8)	0.3 (0.0, 1.3)	0.5 (0.0, 2.4)	1.0 (0.0, 3.6)	0.4 (0.0, 2.1)
Other Wild Bird	0.3 (0.0, 1.8)	0.5 (0.0, 2.8)	0.2 (0.0, 1.3)	0.8 (0.0, 4.1)	0.9 (0.0, 4.4)	0.4 (0.0, 3.3)
Water	0.1 (0.0, 0.7)	0.1 (0.0, 0.8)	0.1 (0.0, 0.9)	0.1 (0.0, 0.9)	0.2 (0.0, 2.4)	0.2 (0.0, 1.6)
	2011–2012	**2012–2013**	**2013–2014**	**2014–2015**		
Supplier A	14.8 (0.3, 32.1)	9.8 (0.0, 28.0)	9.9 (0.0, 27.7)	30.1 (14.0, 46.2)		
Supplier B	15.7 (0.9, 30.9)	10.6 (0.1, 25.1)	17.2 (2.0, 31.9)	10.8 (0.1, 25.9)		
Supplier Other	15.3 (0.0, 41.2)	5.2 (0.0, 21.3)	17.2 (0.0, 39.4)	9.9 (0.0, 30.7)		
Duck	0.1 (0.0, 0.8)	0.1 (0.0, 0.8)	0.1 (0.0, 0.5)	0.1 (0.0, 1.1)		
Turkey	0.3 (0.0, 2.9)	0.3 (0.0, 2.7)	0.1 (0.0, 1.3)	0.4 (0.0, 3.5)		
Spent Hen	1.2 (0.0, 10.0)	0.9 (0.0, 9.1)	1.3 (0.0, 10.7)	0.5 (0.0, 4.4)		
Cattle	42.5 (2.1, 59.3)	59.1 (1.0, 76.8)	39.4 (1.3, 55.4)	34.9 (1.2, 49.9)		
Sheep	2.8 (0.0, 34.6)	4.0 (0.0, 58.4)	4.6 (0.0, 36.7)	2.8 (0.0, 31.3)		
Dog/Cat	1.6 (0.0, 15.0)	0.8 (0.0, 8.3)	2.6 (0.0, 20.5)	0.7 (0.0, 6.2)		
Wild Water Bird	2.0 (0.0, 6.7)	1.8 (0.0, 8.1)	1.4 (0.0, 5.7)	5.1 (0.0, 13.3)		
Other Wild Bird	3.0 (0.0, 11.8)	6.8 (0.0, 17.6)	6.2 (0.0, 14.1)	4.3 (0.0, 16.5)		
Water	0.8 (0.0, 6.6)	0.7 (0.0, 5.8)	0.1 (0.0, 1.3)	0.3 (0.0, 2.4)		

Table 12: The estimated percentage of human cases of campylobacteriosis in the Manawatu attributable to each source for each year from July 2005– June 2015. 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 Other are the poultry suppliers.

4.6 Dynamic modelling

4.6.1 Dynamic Island model

Figure 12 shows the output from the dynamic Island model, displaying the attribution to poultry, ruminant, water, and other sources over the 11 year period. In 2015 there were fewer cases than in 2013 and 2014 overall, and the distribution of cases across the year was more markedly peaked in the summer. Figure 13 shows the updated number of cases attributed to poultry and ruminants including 95% credible envelopes. The similarity in the curves is largely due to the changes in total number of cases over time, though there are some differences, such as the increased poultry attributed cases during summer 2013/2014. For the duration of 2015, however, the attribution of cases to poultry and ruminants has been similar each month.

4.6.2 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 in rural areas, whereas poultry are the most important reservoir for cases residing in urban areas (Figure 14).

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. We can also see that the increase in cases in 2013 was seen in both urban and rural areas, and that 2014 and 2015 are have lower counts in comparison.

There is a clear difference in the attribution of the 2015 rural and urban cases in terms of the proportions attributed to poultry and ruminants, as seen in Figure 15. This is similar to what was seen in 2013, but differs from 2014, where the attribution of rural and urban cases were quite similar (Figure 14).

In addition to the asymmetric island model, we also assessed urban and rural attribution before and after the intervention in the poultry industry using the new R package `sourceR`. Figure 16 shows the attribution to the three

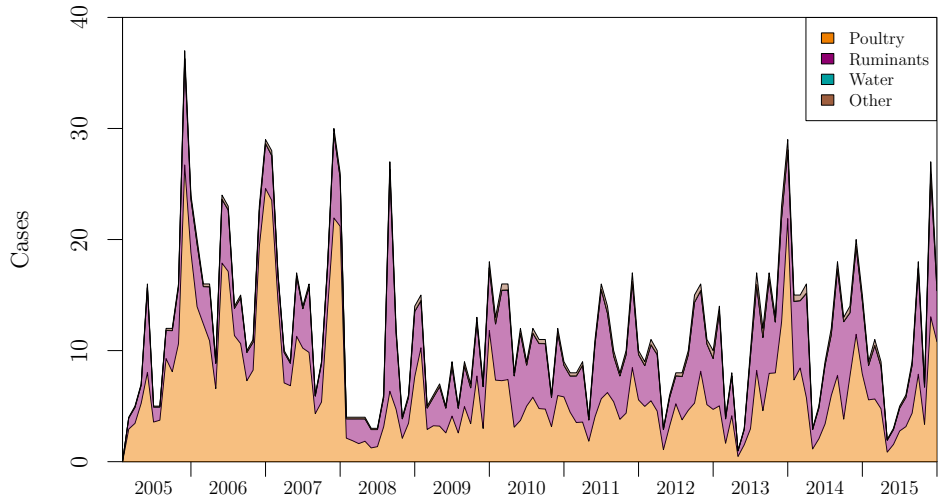

Figure 12: Estimated number of human cases per month attributed to each source by the dynamic Island model from 2005 to 2015. Colours indicate the source the cases are attributed to: poultry (orange), ruminants (purple), water/environmental (blue) and other (brown).

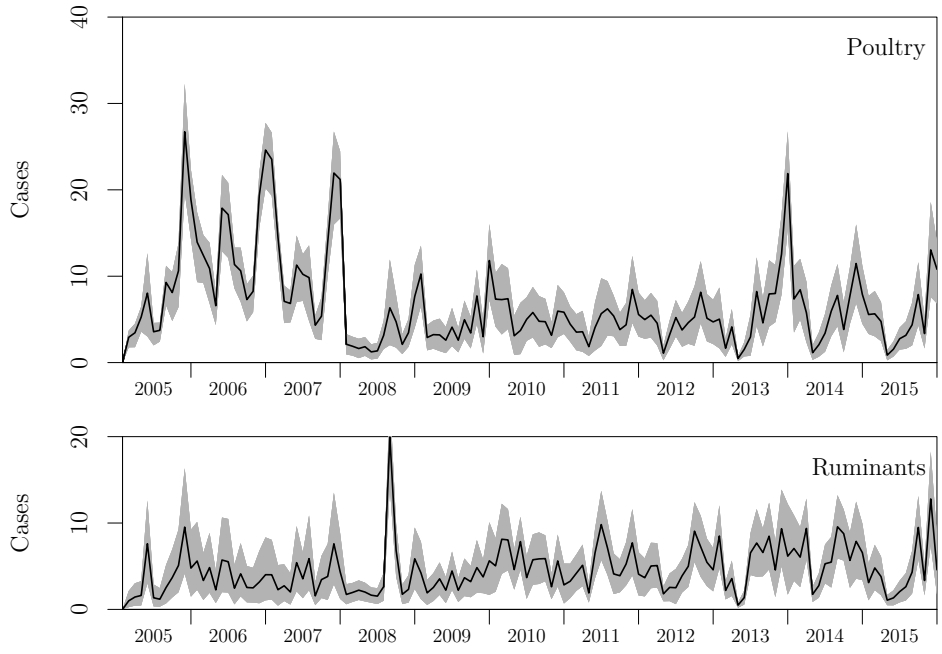

Figure 13: Estimated number of human cases per month attributed to poultry and ruminants by the dynamic Island model with 95% credible intervals from 2005 – 2015.

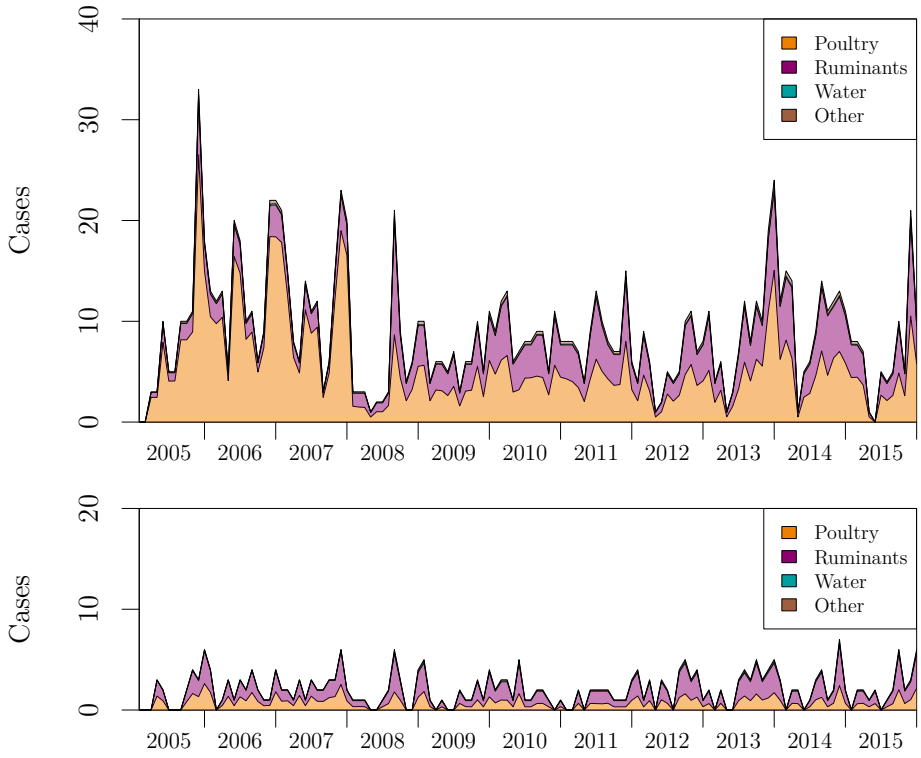

Figure 14: Urban and rural cases 2005–2015: Estimated number of human cases attributed to each reservoir per month by the dynamic Island model.

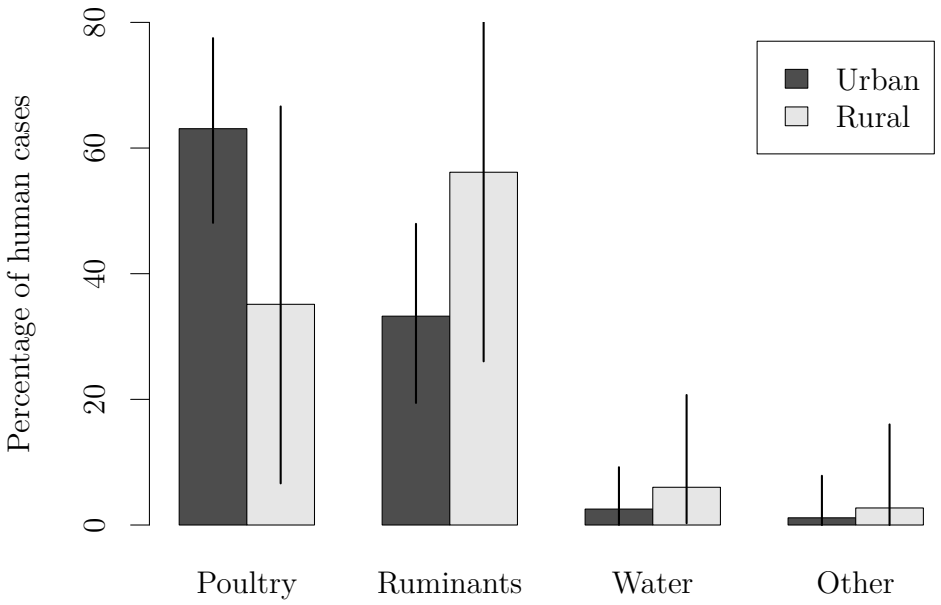

Figure 15: Urban and Rural cases 2015: Estimated proportion of human cases attributed to each reservoir determined by the Island model.

main source groups (total poultry, total ruminants, other) for urban and rural dwellers for 2005–2007 and 2008–2015. Urban dwellers from 2005–2007 were much more likely to be attributed to poultry than ruminants, whereas rural dwellers in the same period were relatively equally distributed between the two main sources. From 2008 onwards, urban cases are equally distributed between poultry and ruminant sources, while rural cases are more likely to be attributed to ruminants.

The `sourceR` package also allows the estimation of genotype effects, which measure whether types are overrepresented among the human cases compared to other types, based on the distribution seen of those types on the sources. Figure 17 presents the types with largest q-values. Interestingly the top two sequence types, ST-474 and ST-38, both belong to the same clonal complex (CC-48), and both *C. jejuni* and *C. coli* types feature, as does the new poultry-related strain ST-6964.

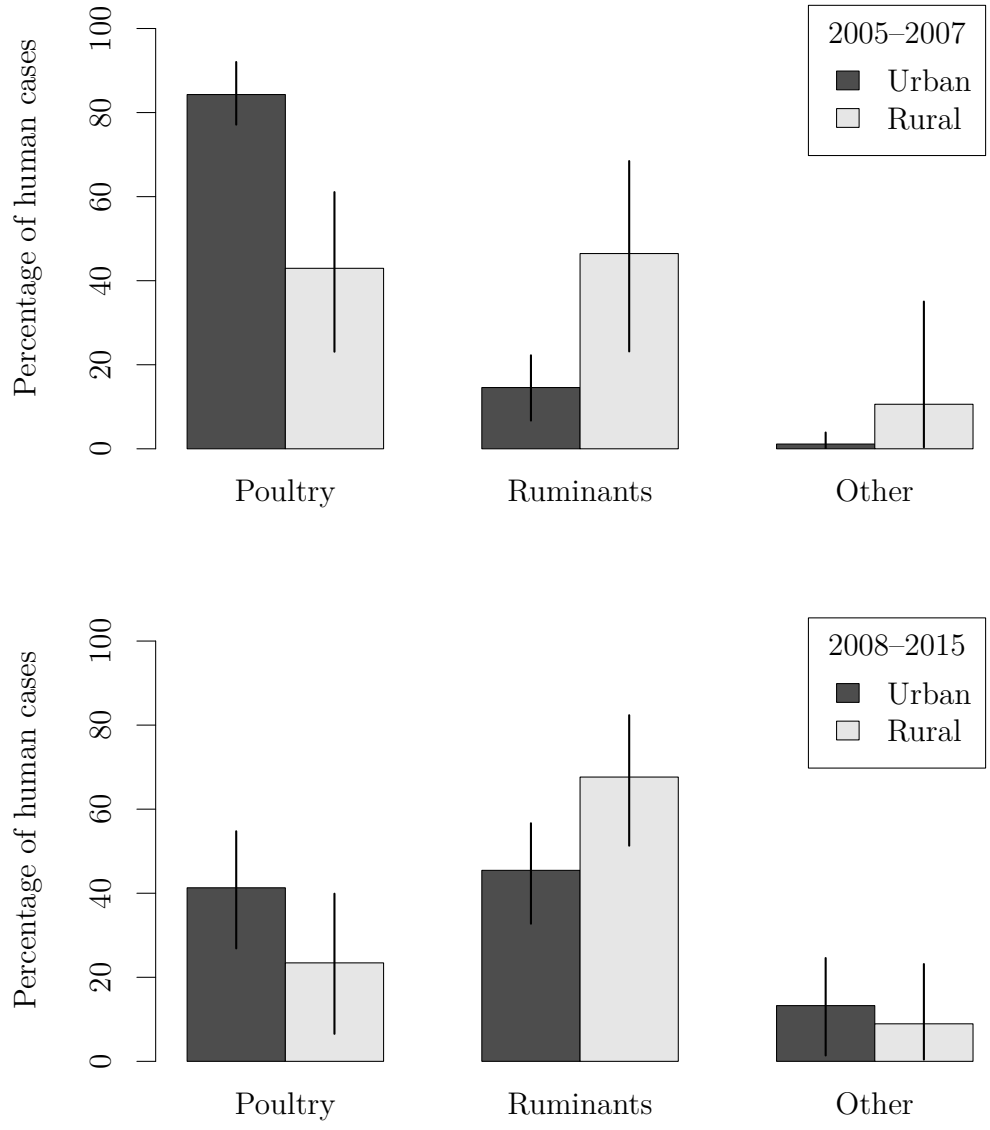

Figure 16: Estimated proportion of urban and rural cases attributed to each reservoir 2005–2007 (top) and 2008–2015 (bottom) as determined using `sourceR`.

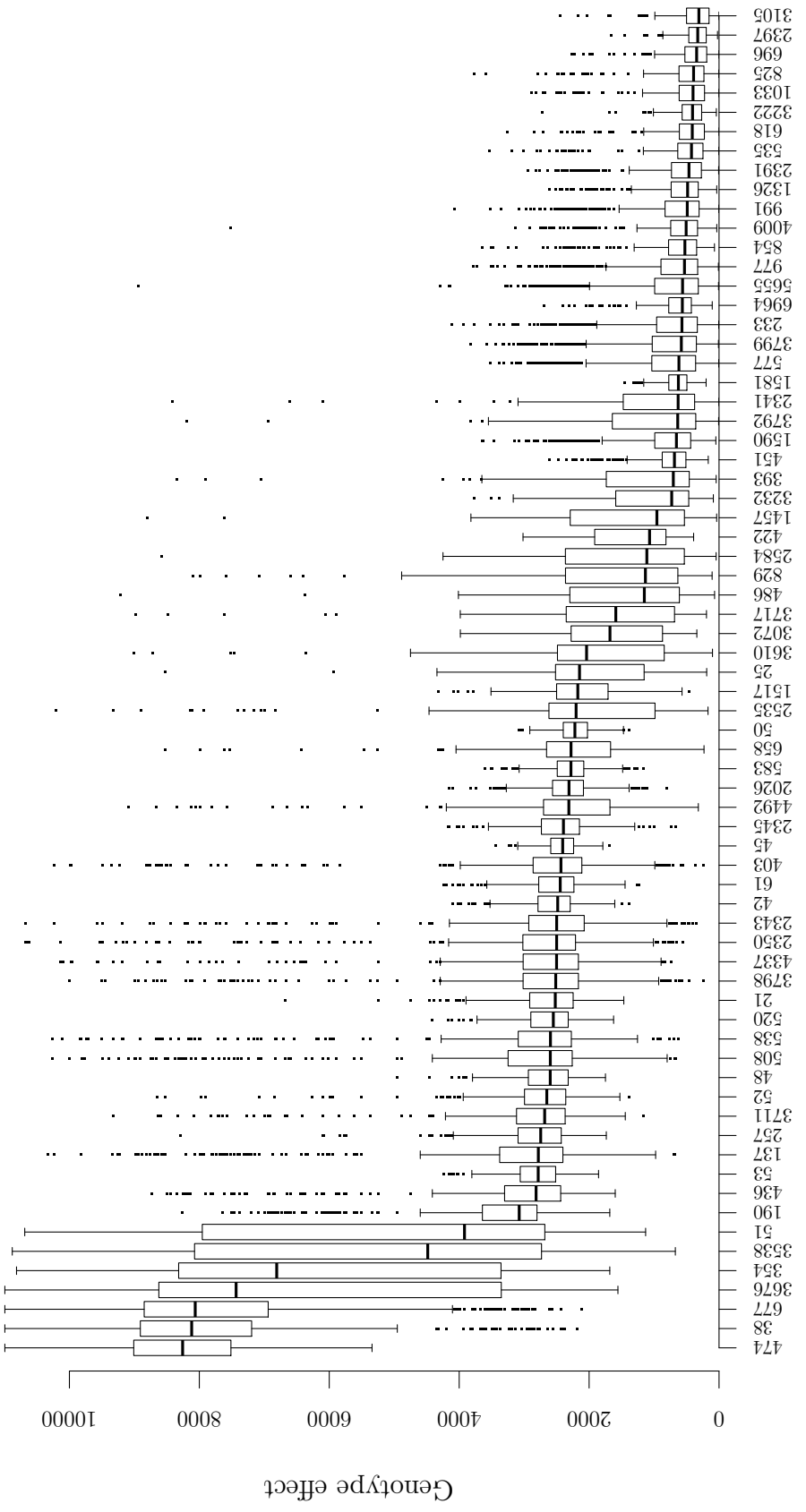

Figure 17: Genotype effects from the `sourceR` model, with larger values indicating that the sequence type is overrepresented among human cases compared to other types.

4.7 Statistical modelling of case rates in District Health Boards over time

Smooth trends and seasonal components through time for case rates per 100,000 population per District Health Board are shown in Figure 18 and Figure 19. It is clear that the trend differs across district health boards, with the more urban DHBs generally having a larger reduction following the intervention in the poultry industry in 2007/2008, with South Canterbury being the exception, being reasonably rural (29%) yet showing a large reduction in case rates.

MidCentral DHB doesn't show a particularly strong reduction following the intervention, though it should be noted that the strong seasonal component in MidCentral from 2005–2007 reduces significantly in amplitude after 2008 which masks the magnitude of the reduction. Some of the mostly rural smaller DHBs (Northland, Tairawhiti, West Coast and Wairarapa) show little evidence of a reduction, supporting the hypothesis that the reduction primarily affected urban areas. Thus, while the number of cases through time in the MidCentral region does not show the same magnitude of reduction post-intervention compared with NZ as a whole, this may in part be due to the higher rural population (18.4% versus 13.8%).

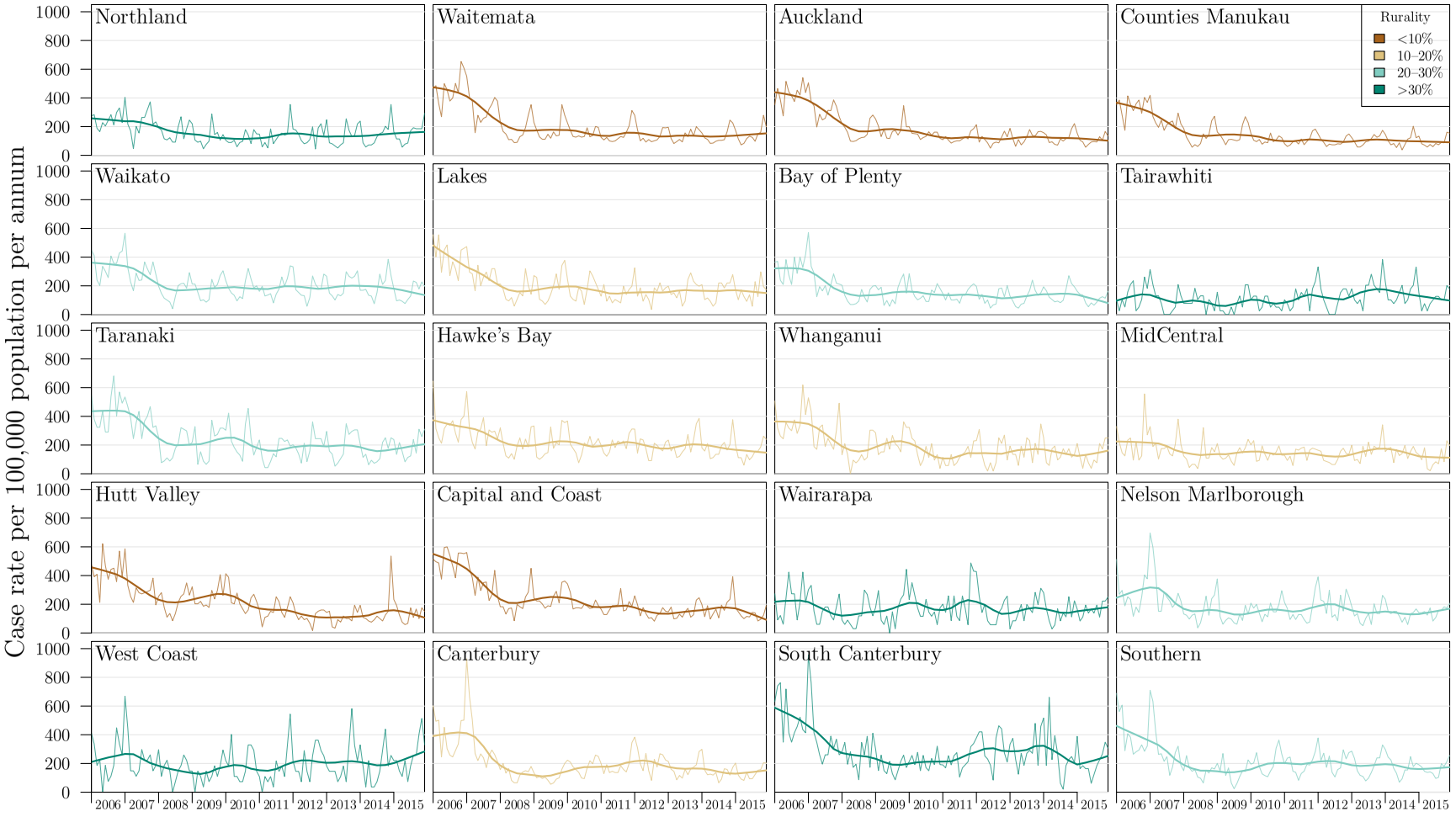

Figure 18: Comparison of trends in case rates per 100,000 population across district health boards. Light lines are the data, and heavy curves represent smooth trends after accounting for seasonality (Figure 19). Colours represent the percentage of rural population per DHB.

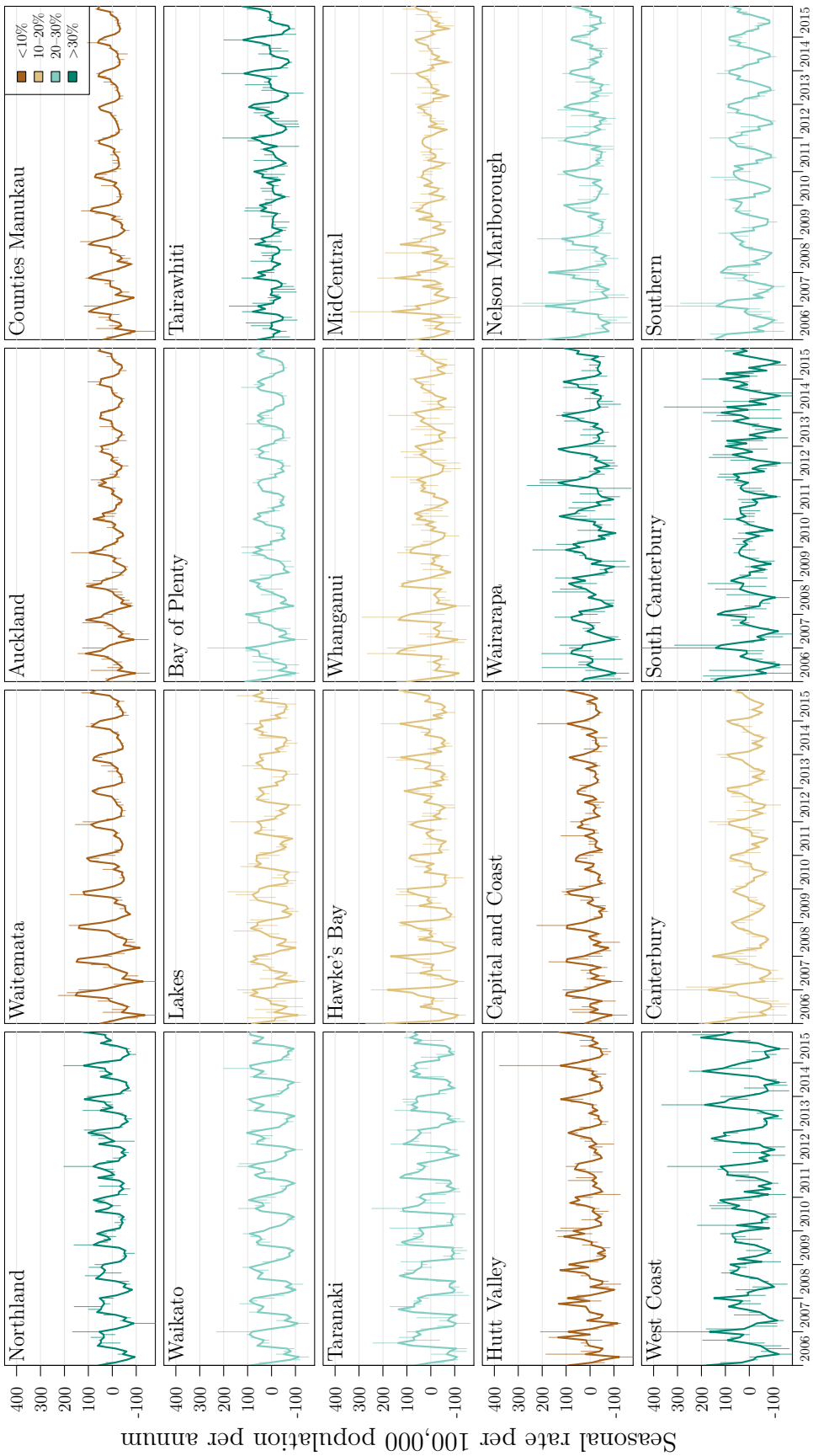

Figure 19: Comparison of seasonality in case rates per 100,000 population across district health boards. Vertical lines represent residuals after smooth trends (Figure 18) and seasonality (heavy curves). Colours represent the percentage of rural population per DHB

Figure 20 summarises the urban and rural population by DHB according to the 2013 census. It is clear that the predominantly urban DHBs are by far the largest, thus total case rates in New Zealand tend to the trend exhibited in those DHBs (Figure 21), rather than the trend in the smaller, more rural DHBs. The trend for MidCentral DHB (Figure 21) shows a lower case-rate prior to the poultry intervention and a small bump in 2013 which may in part be due to a change in the ELISA used around this time, which was observed to be less specific compared to the ELISA used before 2013 and in 2015. The change in seasonality in MidCentral following the poultry intervention is more marked than in New Zealand as a whole.

To further assess the effect of rurality on case rates, the cases for MidCentral were divided into urban and rural subgroups and trends through time were compared (Figure 22). A more marked decline in 2007–2008 is seen in the urban rates, decreasing from approximately 80 to 50 cases per 100,000 population per annum, while the rural rates remain relatively stable, at around the same level (80 cases per 100,000 population per annum) over time. There is a peak in late 2013 in both urban and rural rates and an interesting change in seasonality, with 2008 and earlier showing marked summer peaks, while from 2010 onwards a marked dip in April/May is noticeable, with the summer peak being consequently more broad. As expected, the rural case rate series has more variation due to the smaller population.

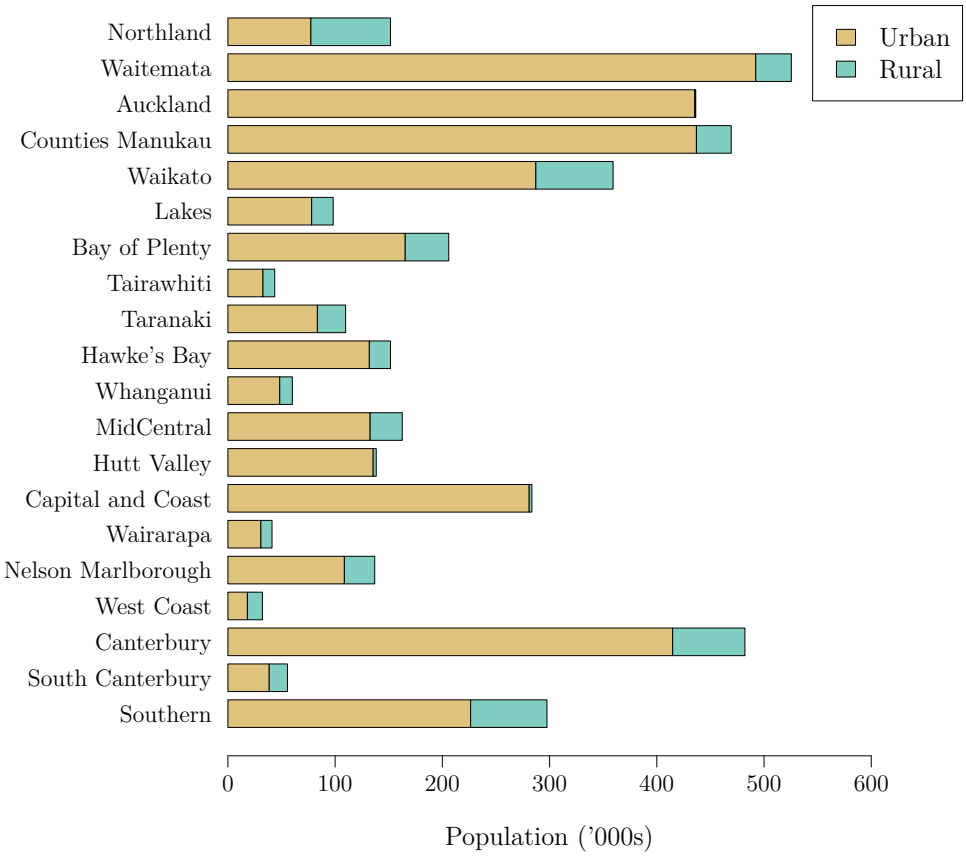

Figure 20: Urban and Rural population ('000s) per district health board based on the 2013 census.

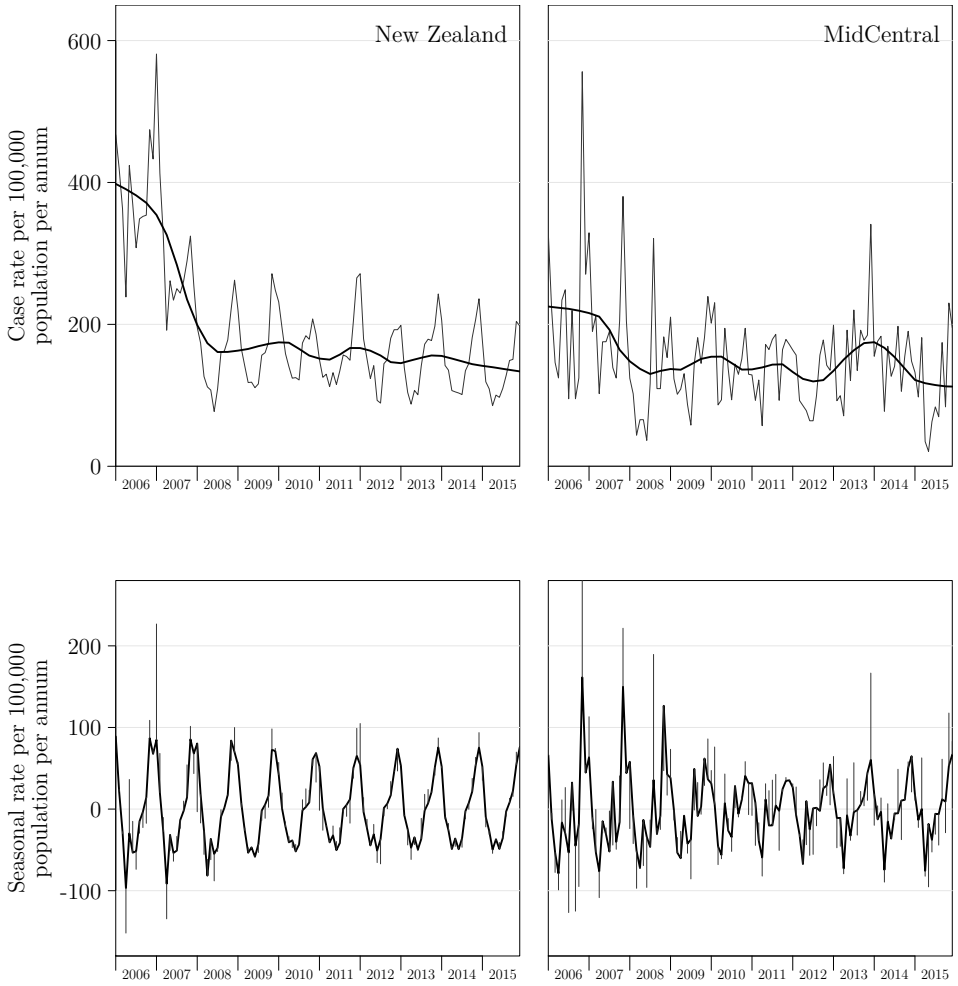

Figure 21: New Zealand (left) and Midcentral DHB (right) campylobacteriosis case rates over time. Rates and smooth trend (top) and seasonality with residuals (bottom).

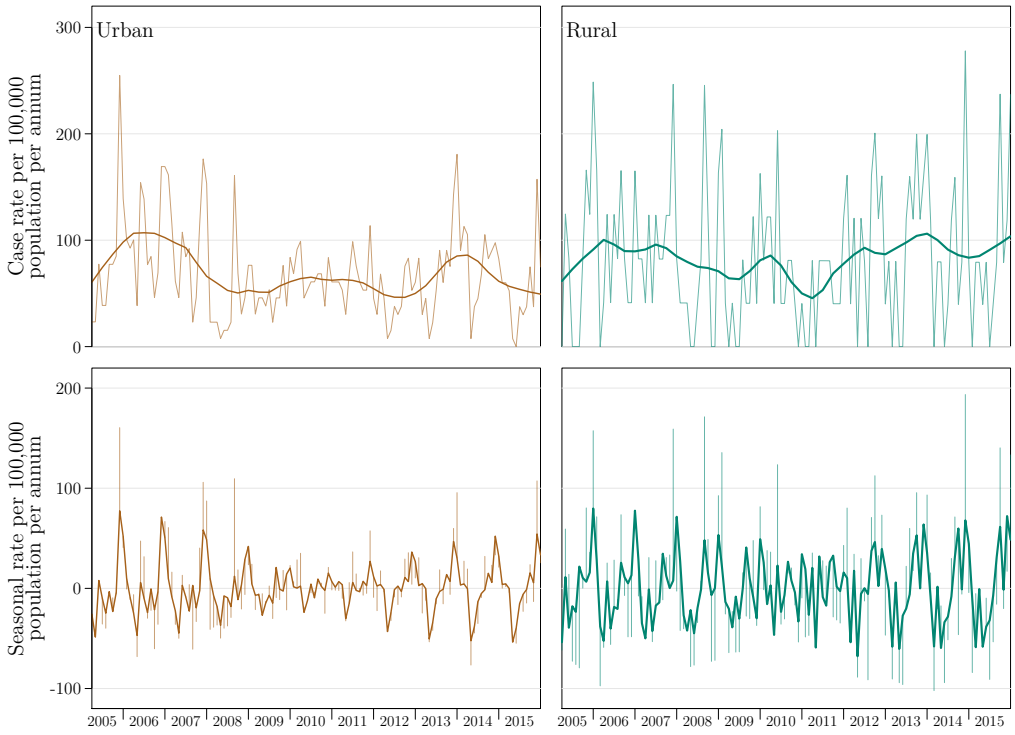

Figure 22: Case rates over time for urban (left) and rural (right) populations within the study region. Rates and smooth trend (top) and seasonality with residuals (bottom).

5 Discussion and Conclusions

This report provides an update of the molecular epidemiology of campylobacteriosis in the Manawatu in 2015 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 December 2013, the pattern of similar attribution to poultry and ruminants observed since 2010 has continued, though this year has seen a slightly higher attribution to poultry compared with ruminants once uncertainty is considered.

The attribution of urban and rural cases in 2015 differs from in 2014, where urban and rural dwellers had a largely similar attribution with both groups being attributed approximately equally to ruminants and poultry. In 2015 the pattern returned to that seen previously where a higher proportion of urban cases are attributed to poultry, while rural cases are more likely to be attributed to ruminants. As noted in previous years, attribution within rural dwellers is typically more stable than within urban dwellers, with most of the variability in urban cases being in the poultry attributed cases.

There has been an increased proportion of *C. coli* isolates from poultry since 2013 compared to previous years. This may in be 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', however we note that *C. coli* STs have been found in the latest round of ruminant sampling which were previously unseen in this source.

In 2014 a new sequence type, ST-6964, emerged in the Manawatu, causing three human clinical cases and being isolated from poultry sources. This ST has quickly become the dominant type found from poultry suppliers A through C, accounting for 31% of all isolates. The type has also been seen in a further 4 human cases, while not being found in other non-poultry sources.

While only a single round of ruminant sampling (i.e. one visit per farm) has been completed as of the end of 2015, we have seen all previously identified sequence types in this round in addition to a number that were previously unseen. Further, new sequence types which have yet to be designated an ST

number in the PubMLST database were found in this round.

A comparison of the case rates of district health boards through time showed significant variability in trend and seasonality, with predominantly urban DHBs showing a large decrease in rates following the intervention in the poultry industry, while those with a higher rural population generally showed less of a decrease. This was also seen when the urban and rural cases within the Manawatu were analysed separately, with rural case rates remaining fairly constant over the period while urban case rates saw a decline post-2007. This is consistent with rural cases being more likely to originate from non-food sources, such as contact with animals or other environmental exposures.

The seasonality of case rates also varied over time, with larger amplitudes prior to the poultry industry intervention. In the Manawatu, the strong summer peak has been replaced by a large April/May dip and a consequently broader summer rise post 2010. Further analyses of cases by district health board, particularly to identify whether the association with rurality is also present within DHBs would require case numbers per month by rurality, which is not available through public data releases.

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[9] http://mepilab.massey.ac.nz/

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