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Haumaru Kai Aotearoa

Source attribution January to December 2016 of human *Campylobacter jejuni* cases from the Manawatu

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Scientific Interpretative 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, 2016.

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 2016 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* and coli samples from animals and humans in the Manawatu sentinel site. These were catalogued and stored at mEpiLab, Massey University.

The 276 primary human samples yielded 196 full MLST allelic profiles. Reservoir attribution modelling revealed that 48% - 70% (credible intervals) of the human cases could be attributed to poultry and 25% - 47% to ruminants. Sequence type ST-6964, which was first discovered in New Zealand in 2014, was again common in 2016 though not the most common, accounting for 14% of all poultry isolates. It was also observed in 14 human cases throughout the year. Of the 287 pooled faecal ruminant samples, 105 were positive for *C. jejuni* and 27 for C. coli. STs not previously seen were identified.

There was a marked increase in notified cases within the Manawatu region in 2016, particularly in the later months. This may in part be due to the change in methodology adopted by MedLab central, which is moved from ELISA testing to confirmation by culture, where the latter is considered more sensitive. 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 2016 of human *Campylobacter jejuni* cases from the Manawatu

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

June 2017

prepared for
the Ministry for Primary Industries

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mEpiLab

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Report: MPI Agreements 17433 and 17509

Source attribution January - December 2016 of human *Campylobacter jejuni* cases from the Manawatu.

June 2017

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 2016. A total of 345 samples were submitted to the mEpiLab in this period, of which 276 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 276 primary samples, 246 were successfully cultured and 194 samples were sequence typed (1 were typed directly from the swab without culturing), yielding 196 MLST allelic profiles[1]. Of these, 169 were *C. jejuni* or *C. coli* that could be linked to EpiSurv data from cases, with (N=25) remaining unlinked.

The new sequence type ST-6964 observed on poultry was again common in 2016 though not the most common, accounting for 14% of all poultry isolates, compared to 30% in 2015. It was observed on carcases from poultry suppliers A, B, and other (both suppliers C and D), and was also observed in 14 human cases throughout the year.

Reservoir attribution modelling revealed that 48–70% of human cases could be attributed to poultry with 25–47% attributed to ruminants in calendar year 2016. 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 2016 showed that urban cases were a little more likely to be attributed to poultry (45–70%) while rural cases were more likely attributed to ruminants (30–70%). As in most years, there were relatively few (48) rural cases with MLST information, so there is significantly more uncertainty in rural attribution estimates. Dynamic attri-

[1]It is possible to have more MLST profiles than samples sequenced, as samples may be a mix of two or more types.

bution 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.

As a consequence of the increasing notification rates in the Manawatu region, particularly during the last few months of the year, a study of the rates of campylobacteriosis per DHB through time suggests that this is mostly isolated to the Manawatu and neighbouring (Whanganui, Wairarapa) regions. This may in part be due to an increase in notifications following the widely publicised waterborne outbreak of campylobacteriosis in Havelock North in August 2016, though we note that once outbreak cases are excluded, there was no evidence of a change in notification rates in the Hawke's Bay, or across New Zealand as a whole. We note that the increase in notification rates in the Manawatu region was predominantly confined to rural areas (case rates increased from 80 to 150 cases per 100,000 population per annum) and was not shown in urban areas (case rates mostly stable at around 80 cases per 100,000 population per annum). Thus, it may represent an actual increase in rural case rates rather than just a change in notification behaviour.

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 2016 to December 2016) 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.

The effect on attribution of human cases within the study region is expected to be less strong, as we report only those numbers for which we generate an MLST sequence in the attribution results, and thus false positives due to varying techniques by MedLab Central are not included. False negatives however may be an issue, as if mEpiLab does not receive a sample, then that sample can not be included in analyses. The above study also included

typing of all *Campylobacter* isolates from MedLab Central over the same period, allowing us to assess false negatives during this period. A summary of these results has been included in this report.

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[2] (AsureQuality). While other researchers have

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

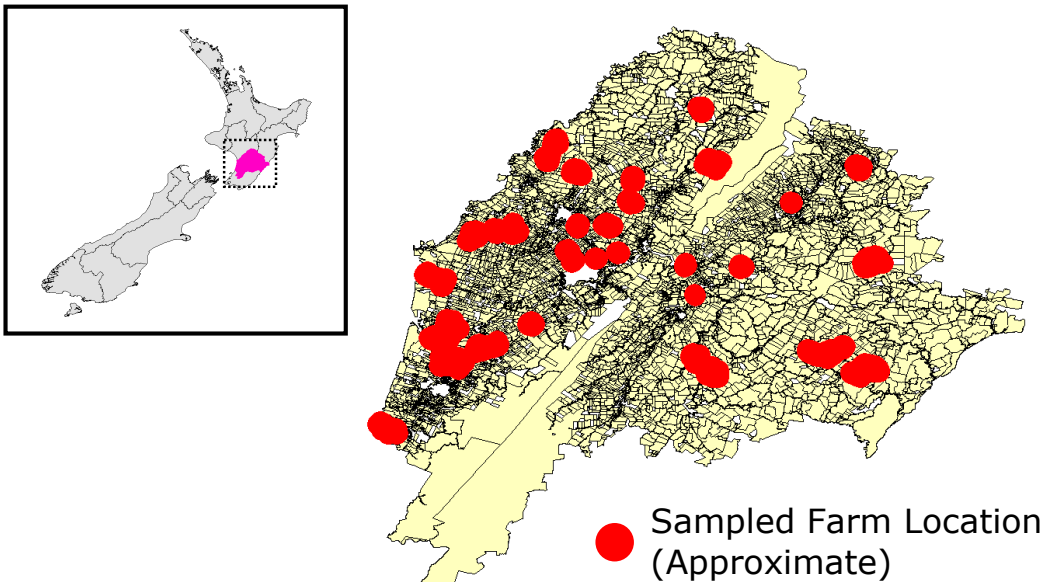

Figure 1: Approximate location of sampled ruminant farms.

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 and twice in 2016 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 Use of whole genome sequencing

Relevant typing methodologies are constantly evolving for all microorganisms, with an increasing demand for high resolution data in microbiological studies and epidemiological modelling. Although the MLST scheme-based methodology for molecular typing of *Campylobacter* species is still the currently accepted gold standard, the global trend is towards the use of whole genome sequencing (WGS) to identify and classify *Campylobacter* isolates. In anticipation of these future demands on epidemiological surveillance data, typing data from ruminant sources in the latest round of sampling for MPI extension contract 17509 was generated using WGS. mEpiLab researchers have vast experience in the study of *Campylobacter* by WGS, and a close relationship with New Zealand Genomics Limited which facilitates our ability to obtain and analyse this data. WGS data is also backwards compatible with MLST typing. To date, 41 of the MLST-types from ruminant samples have been derived from whole genome data. We intend to produce an increasing proportion of all molecular typing results from future surveillance efforts by the use of WGS, thus making the data from current studies future-proof in a changing research environment.

3.1.7 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 2016 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[3] 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.

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

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 2016 were calculated using the Asymmetric Island model as described elsewhere [11, 5, 9]. A model was then run for all the preceding 11 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, 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

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

where

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

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 2016. The PopArt software package[4] [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 note that there has been an increase in case notifications in the Manawatu region in 2016, particularly in the last few months. This may be due to several reasons, one of which might be that the notification rates increased following the widely publicised outbreak of campylobacteriosis in Havelock North in August 2016. To assess this, we include analyses of monthly case rates from each District Health Board (DHB) in New Zealand from 2012–2016.

Numbers of cases per month by DHB were retrieved through the New Zealand Public Health Observatory (for cases from 2012–2014) and from the Monthly

[4] http://popart.otago.ac.nz

Public Health Surveillance reports[5] (ESR Ltd) for 2015–2016 cases.

To compute rates, population counts per meshblock were obtained from Statistics New Zealand[6] 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`[7] 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.
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.

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

4 Results

4.1 Human samples

4.1.1 Human sample information

A total of 345 samples were submitted to the mEpiLab in this period, of which 276 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 276 primary samples, 246 were successfully cultured and 194 samples were sequence typed (1 were typed directly from the swab without culturing), yielding 196 full MLST allelic profiles[8]. Of these, 169 were *C. jejuni* or *C. coli* that could be linked to EpiSurv data from 169 cases, with the remainder (N=25) being unlinked[9]. Note that the number of EpiSurv reports in Table 1 are obtained from the Monthly Notified Disease Surveillance Reports published by ESR[10], and that these differ from the number of EpiSurv data available to be linked to primary samples, primarily as the regions differ.

Once again the lowest number of cases appears in April. The proportion of submitted samples that can be grown up is higher this year, particularly from March onwards (compare with Tables 2 and 3), most likely due to the move away from ELISA to using culture at MedLab central.

[8] It is possible to have more MLST profiles than samples sequenced, as samples may be a mix of two or more types.

[9] 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.

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

2016					
Month	No. samples	Growth	%positive	EpiSurv reports	%coverage
January	27	21	77.8	24	112.5
February	15	11	73.3	18	83.3
March	13	12	92.3	16	81.2
April	6	6	100.0	10	60.0
May	12	12	100.0	16	75.0
June	19	19	100.0	20	95.0
July	17	16	94.1	19	89.5
August	17	14	82.4	22	77.3
September	24	21	87.5	23	104.3
October	29	27	93.1	32	90.6
November	60	53	88.3	54	111.1
December	37	34	91.9	42	88.1
Total	276	246	89.1	296	93.2

Table 1: Details of *Campylobacter* spp. ELISA positive human samples submitted by MedLab Central in 2016. 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.

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	21	91.3	25	92.0
October	10	8	80.0	12	83.3
November	31	28	90.3	33	93.9
December	23	18	78.3	28	82.1
Total	165	132	80.0	196	84.2

Table 2: 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 3: 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.

4.1.2 Distribution of MLST genotypes of human cases

The proportion of human cases with each ST in 2016 is compared with previous years in Table 4. The previously dominant ST-474 accounted for just 4.6% of cases in 2016. This year the most common ST was ST-45 accounting for 15.8% of cases, with ST-50 and ST-6964 accounting for 10.7% and 7.1% of cases respectively. A handful of types not previously observed in humans were found this year in lower numbers (ST-1075, ST-1243, ST-1586, ST-2230, ST-2256, ST-2357).

The genetic variation of *Campylobacter* isolates from humans was analysed for all samples between 2005 and 2016 (Figure 2). Sequence types that were commonly occurring across most years include ST-45, ST-53, and ST-50.

4.1.3 Genetic variation of ST-474

There is some preliminary evidence from whole genome sequencing work on ST-474 isolates that the poultry intervention in 2007/2008 may have eliminated the dominant genetic branch of ST-474. We attributed human isolates

ST	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016
21	0.7	1.4	1.0	2.2	0.9	0.7	3.9		3.3	1.3	3.2	0.5
25		0.5					0.8	0.9		0.6	0.8	0.5
38	2.8	1.9	3.0	3.3	1.9		1.6	0.9	1.3	1.3		1.5
42	3.5	2.9	4.0	4.4	3.8	3.0	4.7	5.1	4.0	5.2	5.6	6.1
45	6.3	7.7	11.6	6.7	10.4	11.2	12.5	13.7	12.7	8.4	18.4	15.8
48	5.6	11.5	6.0	3.3	5.7	4.5	6.2	6.8	4.0	0.6	1.6	1.5
50	4.2	2.4	8.5	7.8	6.6	13.4	8.6	7.7	5.3	10.3	4.8	10.7
53	9.2	4.3	4.0	14.4	4.7	8.2	7.0	7.7	7.3	7.7	2.4	5.6
61	3.5	2.4	3.0	5.6	4.7	6.0	4.7	6.0	5.3	7.1	5.6	5.1
190	7.7	4.3	1.5	7.8	0.9	2.2	3.9	6.0	6.7	0.6	4.8	2.6
227							0.8					0.5
354	1.4	3.3	7.5	4.4					1.3	2.6	1.6	0.5
403		0.5		1.1	0.9	0.7			1.3			0.5
422			1.5	1.1		3.0	0.8	0.9	1.3	3.2	0.8	1.5
436	0.7	0.5	1.0	5.6	0.9	2.2	1.6	1.7	1.3	1.9	1.6	0.5
474	24.6	36.4	24.6	18.9	29.2	11.2	5.5	4.3	3.3	3.2	4.8	4.6
486											2.4	3.6
508								0.9		3.9	0.8	0.5
520	2.8	1.4	1.0		0.9	3.0	8.6	2.6	1.3	3.9	2.4	1.0
583	0.7	1.0	3.5		5.7	3.7	2.3	0.9	5.3	1.3	4.0	6.1
618						0.7						2.0
825									0.7			1.0
1075												0.5
1243												0.5
1517	0.7	1.0	0.5		0.9	0.7	1.6	0.9	2.0	1.9		1.5
1586												0.5
2026	2.1	2.4	1.5	2.2	2.8	0.7	2.3	0.9	2.0	2.6	2.4	2.6
2230												0.5
2256												0.5
2341							0.8					0.5
2345	0.7	1.4			0.9		0.8		4.0	5.8	2.4	4.6
2350		1.0			0.9		0.8			1.3		0.5
2357												0.5
3072		0.5	1.0	1.1		0.7		0.9		0.6	2.4	0.5
3105								0.9			0.8	0.5
3538			0.5		0.9	0.7	1.6	2.6	0.7		0.8	1.5
3676		0.5	1.0	1.1	1.9	0.7		1.7	0.7	1.3		0.5
3711			1.0		0.9	3.7	2.3	0.9	0.7	0.6		0.5
3798							0.8		0.7	1.3	0.8	0.5
4009										0.6	0.8	0.5
4337					0.9			1.7	0.7		4.8	1.0
6964										1.9	3.2	7.1
NEW					1.9			0.9		0.6	3.2	2.0
No. samples	142	209	199	90	106	134	128	117	150	155	125	196

Table 4: The distribution of *C.jejuni* and *C.coli* multilocus sequence types in human cases in 2016 compared with the distribution of the same STs in human cases in the preceding years.

of ST-474 using Whole Genome MLST (a typing scheme using $\sim$ 1400 genes, rather than just the 7 housekeeping genes) to sources, with the supplier A

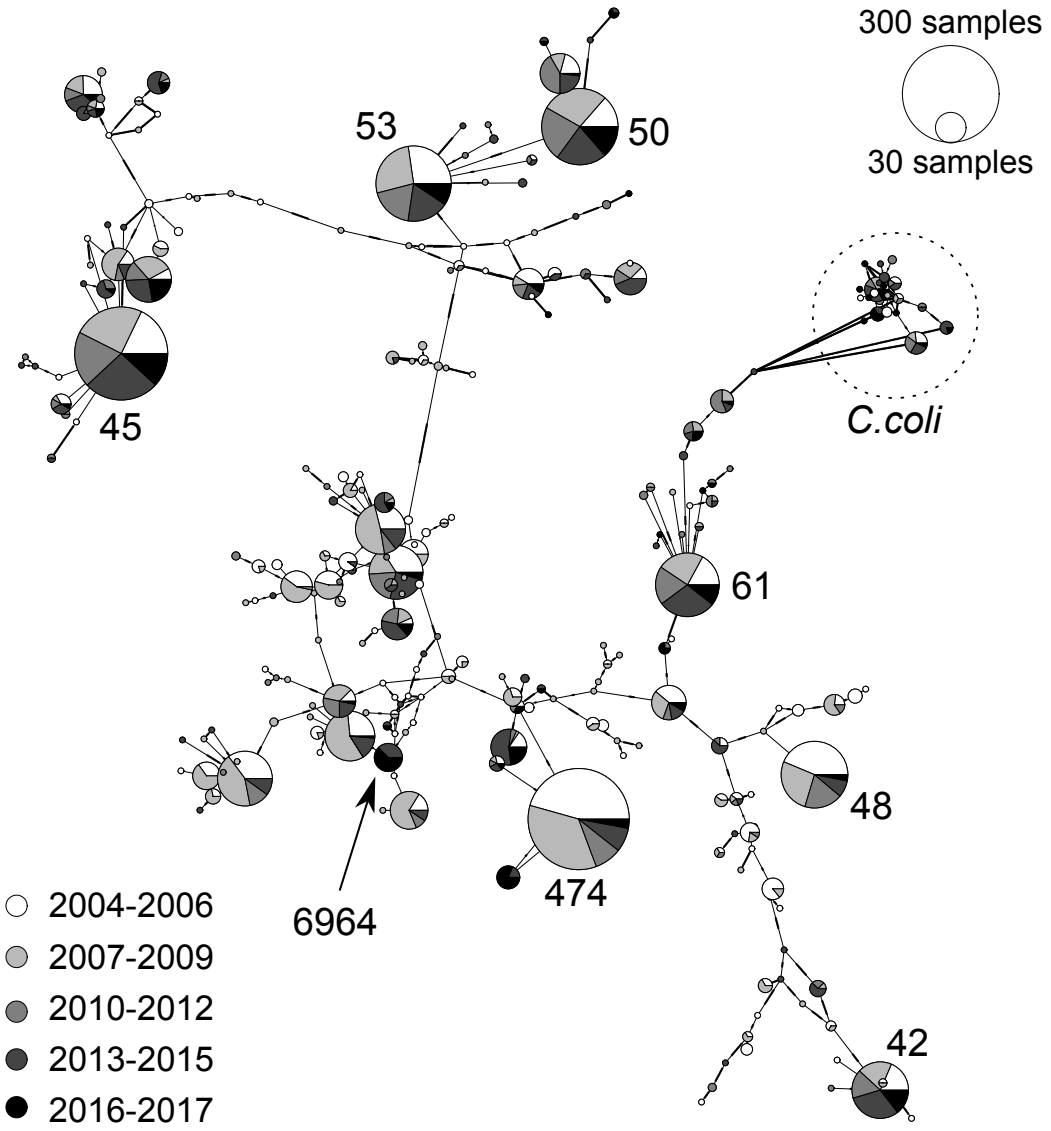

Figure 2: Minimum spanning network of New Zealand Campylobacter isolates from humans between 2005 and 2016. Node size denotes the number of genetically typed isolates belonging to each *Campylobacter* sequence type as specified in the key. Proportions of isolates from individual years of sampling are depicted using progressive shading. Key sequence types are labelled.

source split into pre and post-2008. Figure 3 shows the attribution results, where it can be seen that human cases from before 2008 are almost entirely attributed to supplier A before 2008, with little attribution to supplier A post-2008, suggesting that there is significant genetic variation between ST-474s observed after 2008 on supplier A with those observed before 2008. Similarly, the attribution of human cases post-2008 are less likely to be attributed to supplier A before 2008 compared to supplier A post 2008.

This is preliminary work and verification and validation of the attribution models still need to be done, but it is apparent that ST-474s observed both among humans and on the primary source (supplier A) are different before and after the poultry intervention of 2008. It is currently unknown whether other types show similar temporal variation, or variation among sources when looked at using whole genome sequence data, but it is clear that there is significant potential to utilise the additional level of detail that whole genome sequencing provides in informing source attribution.

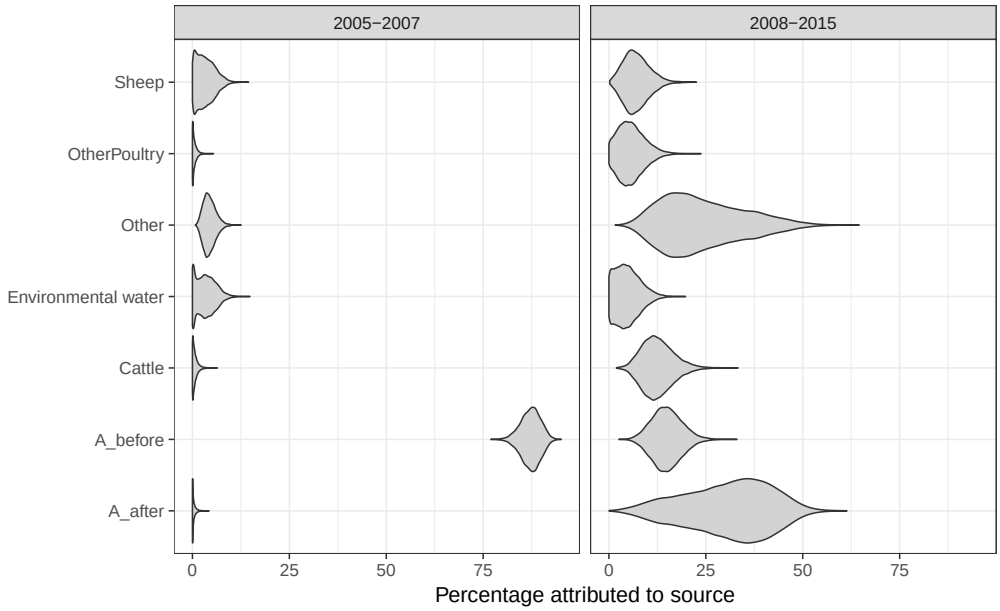

Figure 3: Attribution of human cases of ST-474 from 2005–2007 (left) and 2008–2015 (right) to various sources. Note that the supplier A source has been split in two based on the same time period (before/after 2008).

4.1.4 Analysis of false negative samples

A study was carried out in 2014–2015 of all samples submitted to MedLab Central to assess detection methods. In total 824 samples were collected between 24 April 2014 and 18 January 2015, of which 153 were submitted to mEpiLab in the usual way. Of those submitted to mEpiLab, 115 were confirmed positive for *Campylobacter* (of which 113 were sequenced) while 38 were considered false positives. Of the remaining 671 samples that were not submitted to mEpiLab, 29 were found to be positive for *Campylobacter* (4.3%)[11], with 27 producing an isolates able to be sequenced by MLST. A comparison of the STs from false negatives and true positives was undertaken using permutational analysis of variance. This assesses the variation between the two groups by comparing the ratio of within group variation to residual (between-group) variation, measured using pair-wise dissimilarity of observations across the 7 housekeeping genes. To compare whether the variation observed might be due to chance, the isolates are randomly shuffled between the two groups and the ratio is recomputed, with the proportion of permuted ratios that are larger than the observed ratio giving the P-value. For these data P=0.3, indicating that there is little evidence for a difference in sequence type distribution between false negatives and true positives. Thus, there is likely to be minimal impact on the source attribution models due to missing these samples.

4.2 Poultry samples

4.2.1 Poultry sample information

As planned, 6 poultry samples were taken per month (N=72), of which 70 (97.2%) were *Campylobacter* positive. Of the 70, 68.6% (48) were confirmed as containing *C. jejuni*, and 25.7% (18) were confirmed as *C. coli*. Of these isolates, 9 were mixes containing both *C. coli* and *C. jejuni* STs. All suppliers yielded positive samples. The proportion of carcases positive for *Campylobacter* was 96% for supplier A, 96% for supplier B, and 100% for supplier Other.

[11]Note that some of these may have tested positive by MedLab Central, but were not submitted to mEpiLab due to being out of the study region, as the MidCentral DHB region is larger than the MidCentral public health region that makes up the study area.

Company	Positive	Total	C.jejuni	C.coli	%positive	%2015
Supplier A	23	24	18	9	95.8	100.0
Supplier B	26	27	15	5	96.3	85.7
Supplier Other	21	21	15	4	100.0	93.1
Total	70	72	48	18	97.2	93.1

Table 5: The number of samples positive for presumptive *Campylobacter*, *C. jejuni*, and *C. coli* from each poultry supplier, and the percentage positive in 2016 compared to 2015.

4.2.2 MLST genotypes of poultry isolates

A total of 111 isolates from poultry were successfully MLST typed from 57 of the positive samples yielding 74 unique isolates, with the most prevalent ST being ST-583 followed by ST-2256 and ST-6964. Interestingly, there were a number of MLST profiles from supplier B and 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 2016 (Figure 4). Sequence types that were commonly occurring across all years included ST-45, ST-48, ST-50, and ST-583. The epidemic of ST-6964 that occurred in poultry can be clearly identified within this data.

There was a high prevalence of *C.coli* in 2016, a repeat of the pattern seen in the last two years, with a number of types (ST-825, ST-1581, ST-2256, ST-4009) featuring (Table 7).

There are two main methods used to grow poultry isolates. The first is the standard culture method at 42℃ which has been in use since 2005, and the second is an alternate culture method at 37℃ in an atmosphere with 5% Hydrogen. It may be that the increase in *C. coli* prevalence might be due to more rigorous use of the second method (in addition to the main method) in recent years. To explore this, we compared *C. coli* prevalence across the two methods, grouping the results in two-yearly intervals (Figure 5). As can be seen, there is no suggestion that the alternate method (Method 2) is detecting higher levels of *C. coli* than the main method (Method 1). Both methods have shown increased prevalence in the last 4 years, with the main method (which has been in use since 2005) showing higher prevalence of *C.*

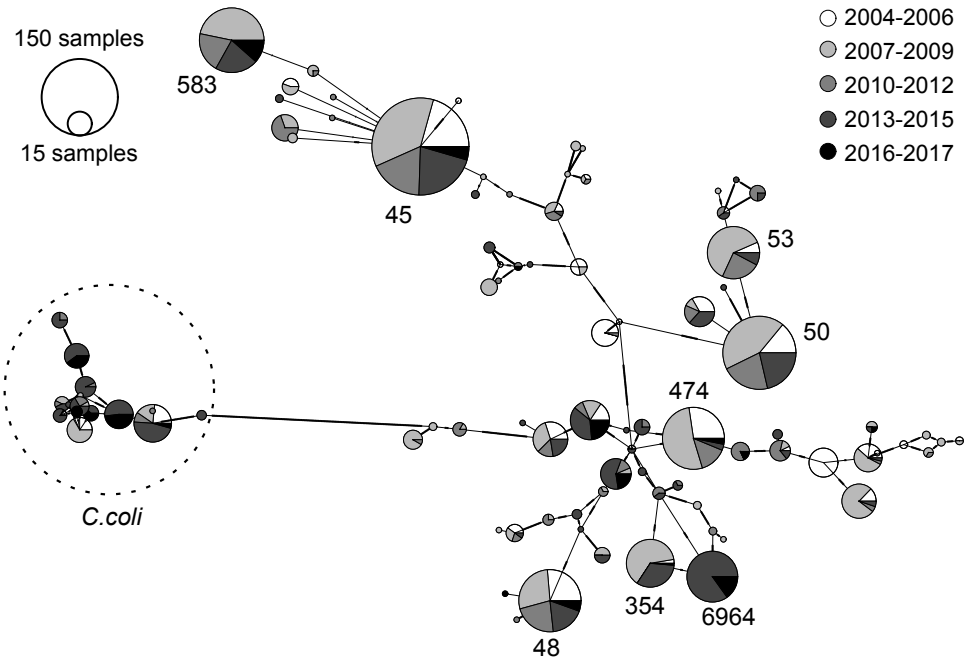

Figure 4: Minimum spanning network of New Zealand Campylobacter isolates from poultry between 2005 and 2016. Node size denotes the number of genetically typed isolates belonging to each *Campylobacter* sequence type as specified in the key. Proportions of isolates from individual years of sampling are depicted using progressive shading. Key sequence types are labelled.

coli compared to the alternate method.

The relatively new type ST-6964 continues to be highly prevalent across suppliers A, B, and Other, appearing in both suppliers C and D in 2016. This sequence type was first observed in 2014, and was also found in 14 human cases in 2016. Table 8 lists the isolates by date and source.

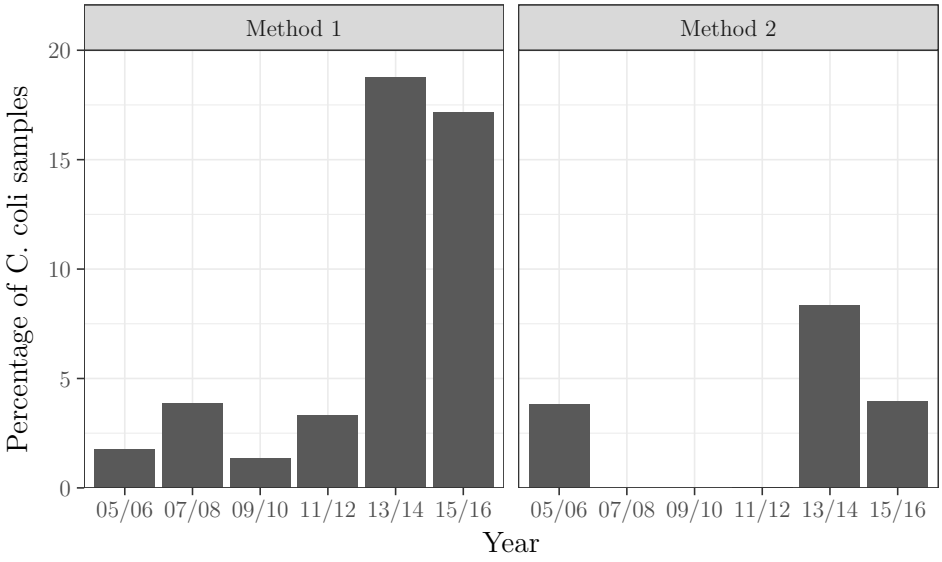

Figure 5: Prevalence of *C. Coli* on chicken carcases over time by the two main culturing methods. Method 1 corresponds to the standard method, while method 2 is the alternate, culturing at 37°C with 5% Hydrogen

	2014			**2015**			**2016**		
ST	A	B	Other	A	B	Other	A	B	Other
21						3.1			
45		9.1	40.0	15.2		12.5	10.0		27.3
48		18.2						22.7	
50		13.6			15.8	18.8			
51				3.0					
52				3.0					
53				6.1		3.1			
257	4.5		4.0						
354	22.7		4.0			3.1			4.5
356	4.5								
422								4.5	
474								9.1	
486								4.5	
535	4.5								
538				6.1					
583	13.6		8.0	6.1		6.2	23.3	9.1	18.2
696				6.1					
739									4.5
825							6.7		
854					15.8	3.1			
1581	9.1	18.2	4.0					4.5	
1590		9.1							
2256				9.1		6.2	23.3	4.5	9.1
2345	4.5			9.1			6.7		4.5
2389						3.1			
2584									4.5
3072					5.3				
3105	36.4			3.0			13.3		4.5
3230			12.0						
3721				3.0					
3792		4.5							
4009		9.1			21.1			13.6	9.1
4159				3.0					
4337					5.3				
6964		18.2	16.0	24.2	36.8	31.2	16.7	13.6	9.1
7767			4.0						
8066			4.0						
8067			4.0						
NEW				3.0		9.4		13.6	4.5
No. samples	22	22	25	33	19	32	30	22	22

Table 6: The distribution of *C.jejuni* and *C.coli* (bold) multilocus sequence types in poultry in 2016 compared with the distribution of the same STs in poultry in the preceding two years (2014, 2015). No. samples refers to the total number of samples examined in each year.

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	40	55.6	4	5.6
2011	72	59	81.9	5	6.9
2012	72	42	58.3	0	0.0
2013	72	42	58.3	22	30.6
2014	72	47	65.3	15	20.8
2015	72	54	75.0	14	19.4
2016	72	48	66.7	18	25.0
Total	1369	748	54.6	117	8.5

Table 7: The number and prevalance of *C. jejuni* and *C.coli* over the years 2005–2016. 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	2		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
Feb 2016	1	1		
Mar 2016	1	1		
Apr 2016		1		
May 2016	1		1	
Jun 2016	3			
Jul 2016	2			
Aug 2016		1	1	
Sep 2016				1
Oct 2016	3			
Nov 2016	2	1		
Dec 2016	1		1	1
Jan 2017	1	2		1
Feb 2017				

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 2016.*

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 6) and the estimated number of *Campylobacter* spp. given the carcase is positive (Figure 7). 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. The trend over the last few years has been largely maintained.

We note that these results may differ to that found in the National Microbial Database (NMD). The difference might be attributed to measuring at retail rather than at the processors which may lead to further contamination, and also potentially due to a more sensitive test used in mEpiLab. The mEpiLab testing utilises duplicate mCCDA plates inoculated with 50uL (spiral plater) or 1mL (spread plate) aliquots of chichen washes, and 100uL (spiral plater) aliquotes of resuspended chicken wash pellets, giving 6 replicates per sample at differing volumes, thus differing resolutions [10]. It may be that this results in a higher proportion of positives, and improved counting resolution than that used for the NMD.

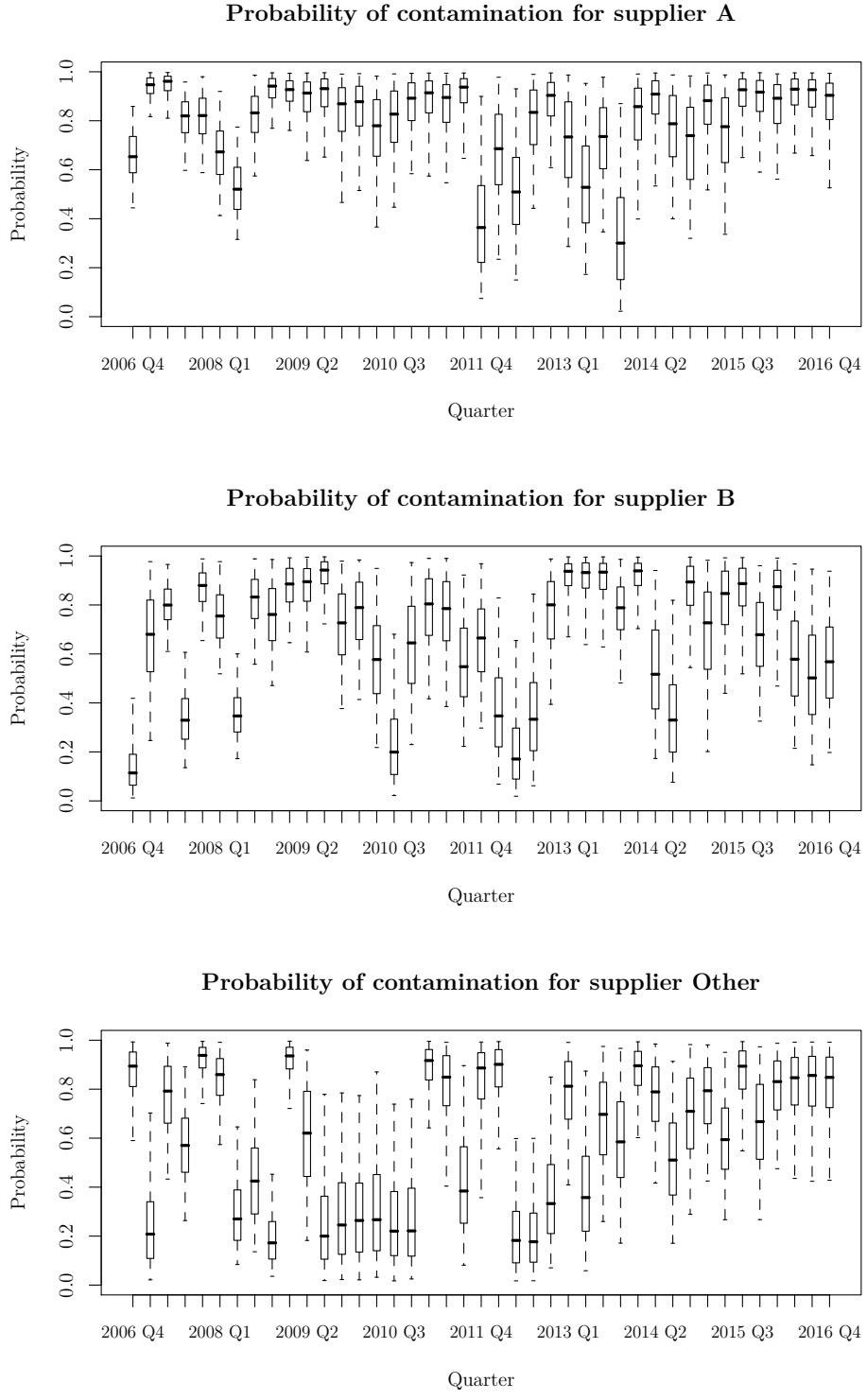

Figure 6: *Campylobacter* on chicken carcases by quarter: probability of contamination for each supplier, showing the median (thick horizontal line), interquartile range (box) and 95% (credible) intervals (dashed lines) of the posterior distribution.

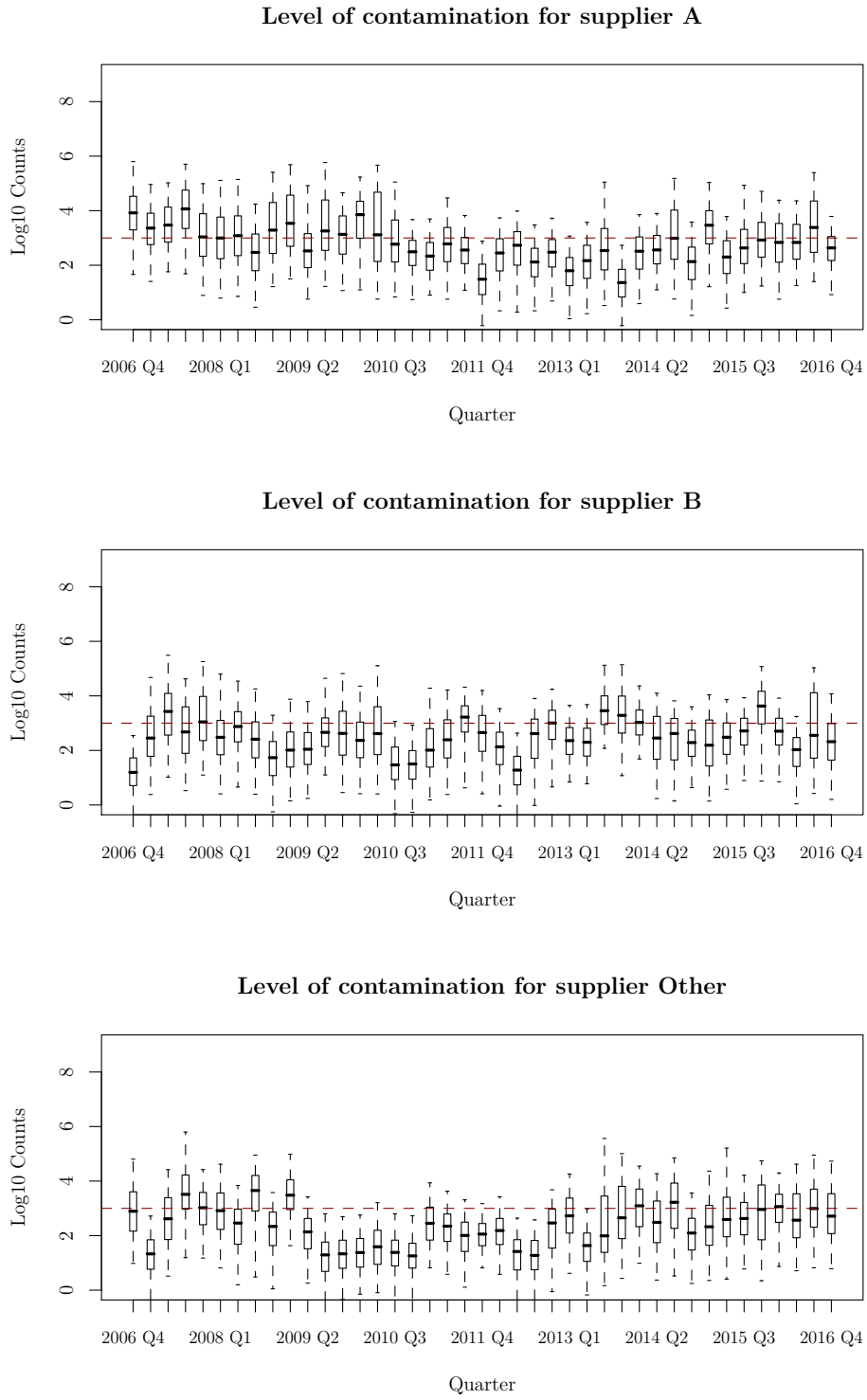

Figure 7: *Campylobacter* on chicken carcases by quarter: estimated level of contamination on positive carcases for each supplier, showing the median (thick horizontal line), interquartile range (box) and 95% (credible) intervals (dashed lines) of the posterior distribution. The dashed horizontal line marks 1000 cfu in the carcase rinsate.

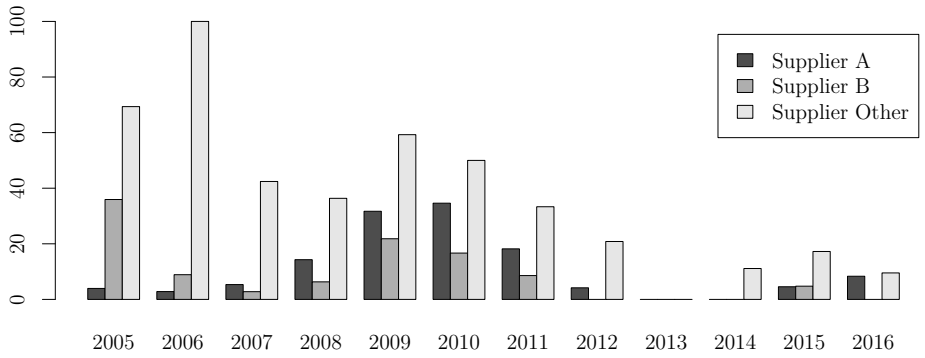

Figure 8: The percentage of chicken carcases with leaking packaging from each supplier from 2005–2016, N=1369.

4.2.4 Packaging of poultry carcases

The proportion of carcases from each poultry supplier with leaking wrappers was examined for the the whole 12 year period (1369 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, with similarly low levels over the last 4 years (Figure 8).

4.3 Ruminant samples

4.3.1 Ruminant sample information

As planned, 1148 ruminant samples were collected from 33 farms (at least 32 samples per farm) across the Manawatu district in 2016. Half of these (16 farms) were taken from dairy, and the other half were taken from sheep (7) or sheep and beef (9) farms. Both beef and sheep samples were taken, when available, on sheep and beef farms. Samples were pooled into sets of 4, yielding 287 pooled samples. Of these, 256 (89.2%) were positive for *Campylobacter*, with 22/22 farms with cattle and 19/19 farms with sheep having at least one positive sample. Of the 256, 41% (105), and 10.5% (27) were confirmed as *C. jejuni* and *C. coli* respectively. Of these isolates, 3 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	141	149	67	14	94.6
Sheep	115	138	38	13	83.3
Total	256	287	105	27	89.2

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 2016.

4.3.2 MLST genotypes of ruminant isolates

A total of 142 isolates from ruminants were successfully MLST typed from 129 of the 256 positive samples, yielding 138 unique isolates, with the most prevalent ST being ST-61 accounting for 32.7% of Cattle and 2.3% of Sheep isolates, followed by ST-42 and ST-50. A comparison of MLST types with types from 2005–2008 is given in Table 10.

Table 10 shows that there is quite a strong overlap in the types observed on cattle and sheep. To assess the extent of this overlap the proportional similarity index was computed between cattle and sheep ST distributions and between two time periods 2005–2008 and 2015–2016. The proportional similarity index is a measure between 0 and 100, where 100 is achieved when

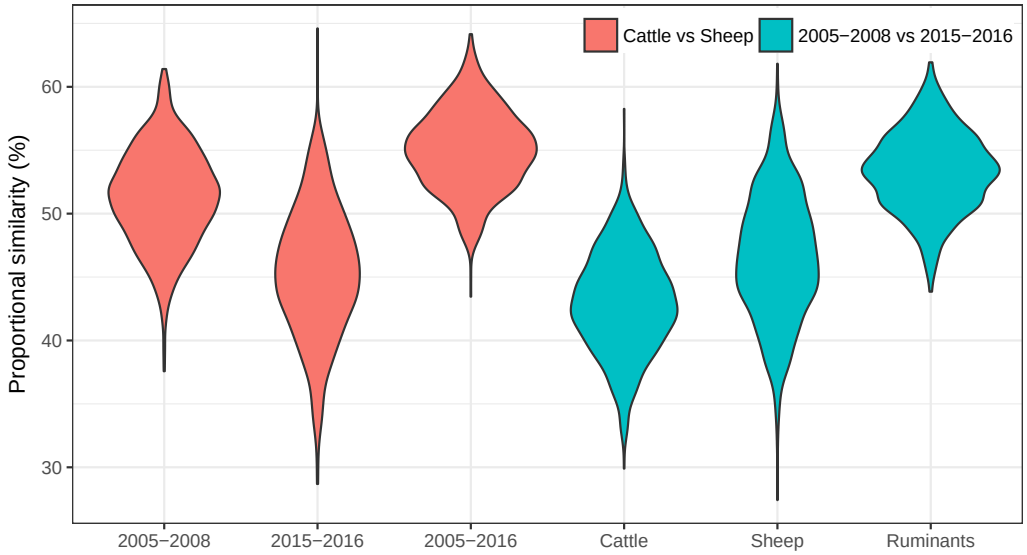

Figure 9: Proportional similarity between *Campylobacter* observed among cattle and sheep faeces from 2005–2008 and 2015–2016. Violin plots represent the uncertainty in this measure. The plots in red compare cattle and sheep within each of the time periods and overall, while those in blue compare the two time periods within each of cattle and sheep and overall.

the proportion of each ST is identical for the two groups being compared. The proportional similarities and their uncertainties are shown in Figure 9. Of interest there is a higher similarity between cattle and sheep within the 2005-2008 year group compared with the 2015-2016 group, and also a higher similarity (though with larger uncertainty) between the time periods within sheep compared with cattle. Overall, the level of similarity is around 50–55%.

The genetic variation of *Campylobacter* isolates from ruminants was analysed for all samples between 2005 and 2016 (Figure 10). Sequence types that were commonly occurring across most years included ST-42, ST-50, and ST-61. A total of 16 sequence types were identified in 2015–2016 that had not been identified in previous sampling rounds including 13 *C. jejuni* types and 3 *C. coli* types. Of these, 12 are known to infect other animals and humans. In addition, there were a number (5 in Cattle) of types in 2016 that as yet have no ST designation, indicating that they have not been submitted to PubMLST previously, accounting for 3.6% of isolates.

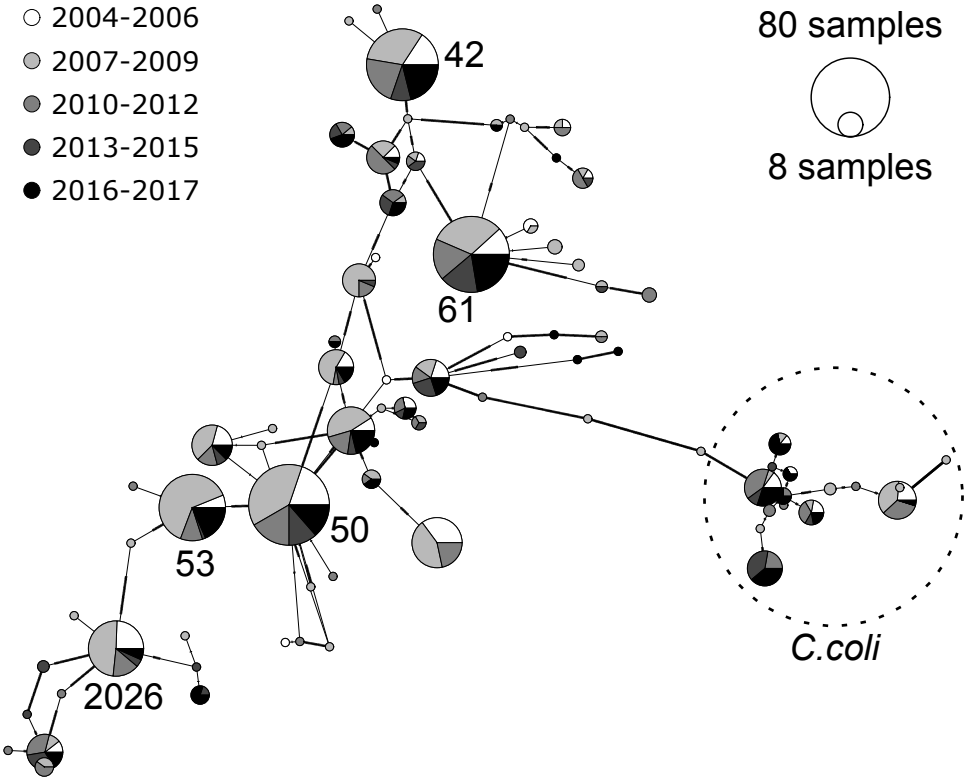

Figure 10: Minimum spanning network of New Zealand Campylobacter isolates from ruminants between 2005 and 2016. Node size denotes the number of genetically typed isolates belonging to each *Campylobacter* sequence type as specified in the key. Proportions of isolates from individual years of sampling are depicted using progressive shading. Key sequence types are labelled.

4.4 Samples available for attribution

There are a total of 4230 isolates with complete *C. jejuni* or *C. coli* allelic profiles available for source attribution, with an additional 88 isolates with partial profiles that could be uniquely assigned to a sequence type from PubMLST, giving 4318 total isolates available for attribution across the period 2005–2016. 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 11. Note that ST-6964 appears near many other poultry and human isolates.

	2005-2008		**2015-2016**			
ST	Cattle	Sheep	Cattle	Sheep	Poultry	Human
21	3.6	1.1	2.0	1.3	0.9	1.6
38	0.7		4.1		0.2	1.7
42	11.4	9.9	11.6	7.7	0.7	4.5
45	1.4	1.1	3.4	5.1	16.4	11.8
50	9.3	15.9	10.2	11.5	7.5	7.8
53	20.0	1.1	6.1	5.1	5.4	6.8
61	6.4	9.3	16.3	9.0	0.7	5.0
190	5.7	5.5		11.5	1.3	4.1
257			3.4	1.3	2.2	2.0
265				1.3		
393		0.5		1.3		0.1
403			0.7			0.4
436	1.4	2.2	0.7	1.3		1.5
474	5.0	1.6	0.7		6.2	15.5
508			1.4			0.5
520	5.0	3.3	1.4	3.8	1.8	2.5
618	0.7	0.5		2.6		0.3
704				1.3		0.1
767			0.7			
829				3.8		0.1
890	0.7		0.7	2.6		
991				2.6		0.1
1107			0.7			
1115	1.4		2.7	1.3		
1517		4.4	4.8	2.6	0.7	1.1
1581			8.8	1.3	3.7	0.8
2026	6.4	11.5	1.4	3.8		2.2
2392		1.1	0.7	2.6		
2536			0.7			
3072	2.9		1.4	7.7	0.3	0.7
3222	5.7	1.1	0.7			0.2
3232		1.6		3.8		0.2
3538			0.7			0.8
3610		1.1	1.4			0.2
3711		1.1	0.7		0.2	0.9
3712				1.3		0.2
3959	0.7		0.7			
4337				1.3	0.3	0.7
4492			0.7			0.2
6997			2.7	1.3		0.1
NEW	0.7	0.5	8.2		1.0	0.7
No. samples	140	182	147	78	986	1673

Table 10: The distribution of *C.jejuni* and *C.coli* multilocus sequence types in ruminants in 2015–2016 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.

	Complete	Imputed	Total
Human	1785	15	1800
Supplier A	391	7	398
Supplier B	369	4	373
Supplier Other	273	3	276
Duck	49	1	50
Turkey	20	4	24
Spent Hen	26	1	27
Cattle	427	14	441
Sheep	320	12	332
Dog/Cat	33	1	34
Wild Water Bird	163	13	176
Other Wild Bird	81	3	84
Water	293	10	303
Total	4230	88	4318

Table 11: Number of complete and imputed *C. jejuni* isolates available for attribution for the years 2005–2016. Imputed isolates are those that have partial allelic profiles that match a unique sequence type in the PubMLST database.

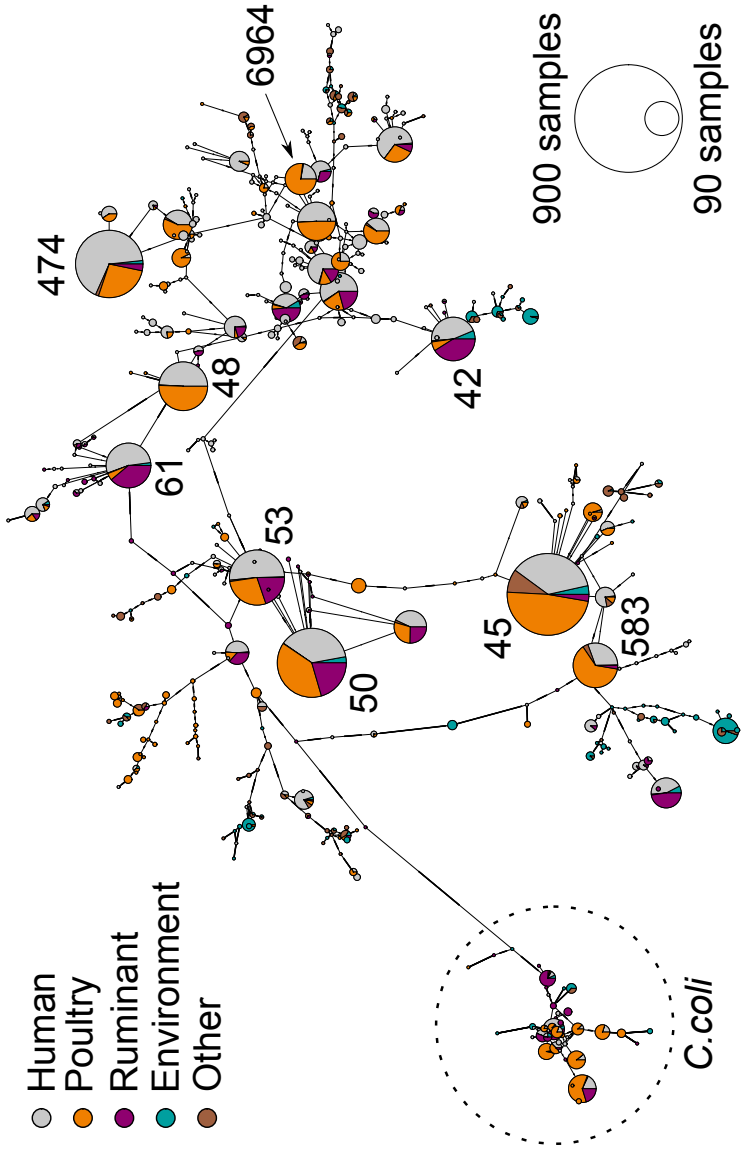

Figure 11: Minimum spanning network of New Zealand *Campylobacter* isolates from humans, ruminants and poultry between 2005 and 2016. Node size denotes the number of genetically typed isolates belonging to each *Campylobacter* sequence type as specified in in the key. Proportions of isolates from different 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 2016 compared to previous years

4.5.1 Source attribution by year

Figures 12 and 13 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 2016. The proportion of cases attributed to poultry is up this year at around 55% with the majority of the rest being ruminant, particularly cattle sources. The number of cases attributed to poultry is still lower than in the pre-intervention period, and the number of cases attributed to cattle is higher, though there is significant uncertainty largely due to being less able to distinguish cattle and sheep based on MLST alone.

Figures 14 and 15 summarises all sources over the 12 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 Figures 16 and 17. The complete table of estimates, including credible intervals is shown in Tables 12 and 13.

Figure 12: 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 2016. Error bars represent 95% confidence or credible intervals.

Figure 13: 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 2016. Error bars represent 95% confidence or credible intervals.

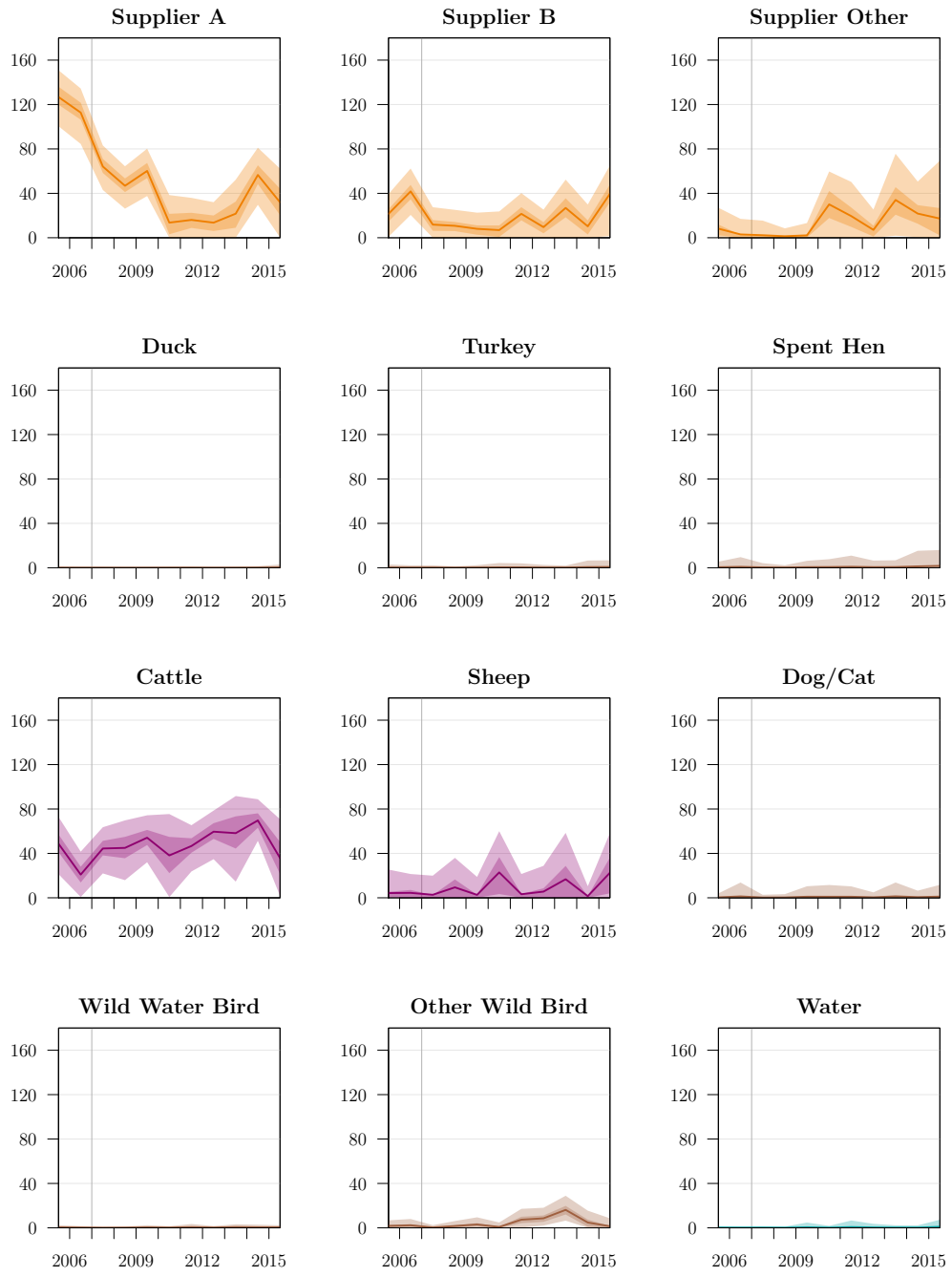

Figure 14: Estimated number of cases attributed to poultry, ruminant and other sources in the Manawatu between July 2005 and June 2016. Years 2006 and 2007 represent the pre-intervention period. Note that July to June is used to ensure the summer peak is centered within each period.

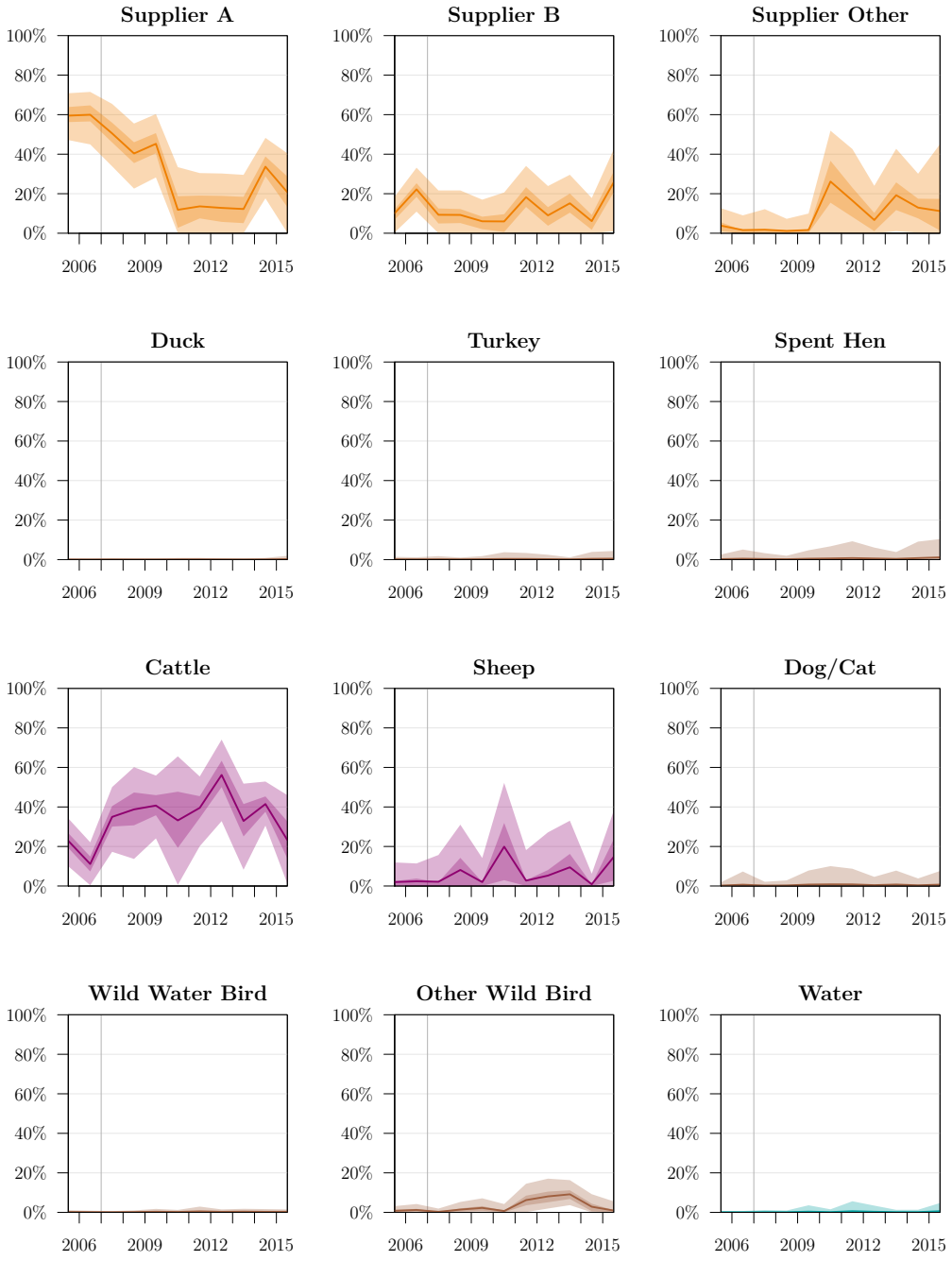

Figure 15: Poultry, ruminant and other source attribution estimates for human cases in the Manawatu between July 2005 and June 2016. Years 2006 and 2007 represent the pre-intervention period. Note that July to June is used to ensure the summer peak is centered within each period.

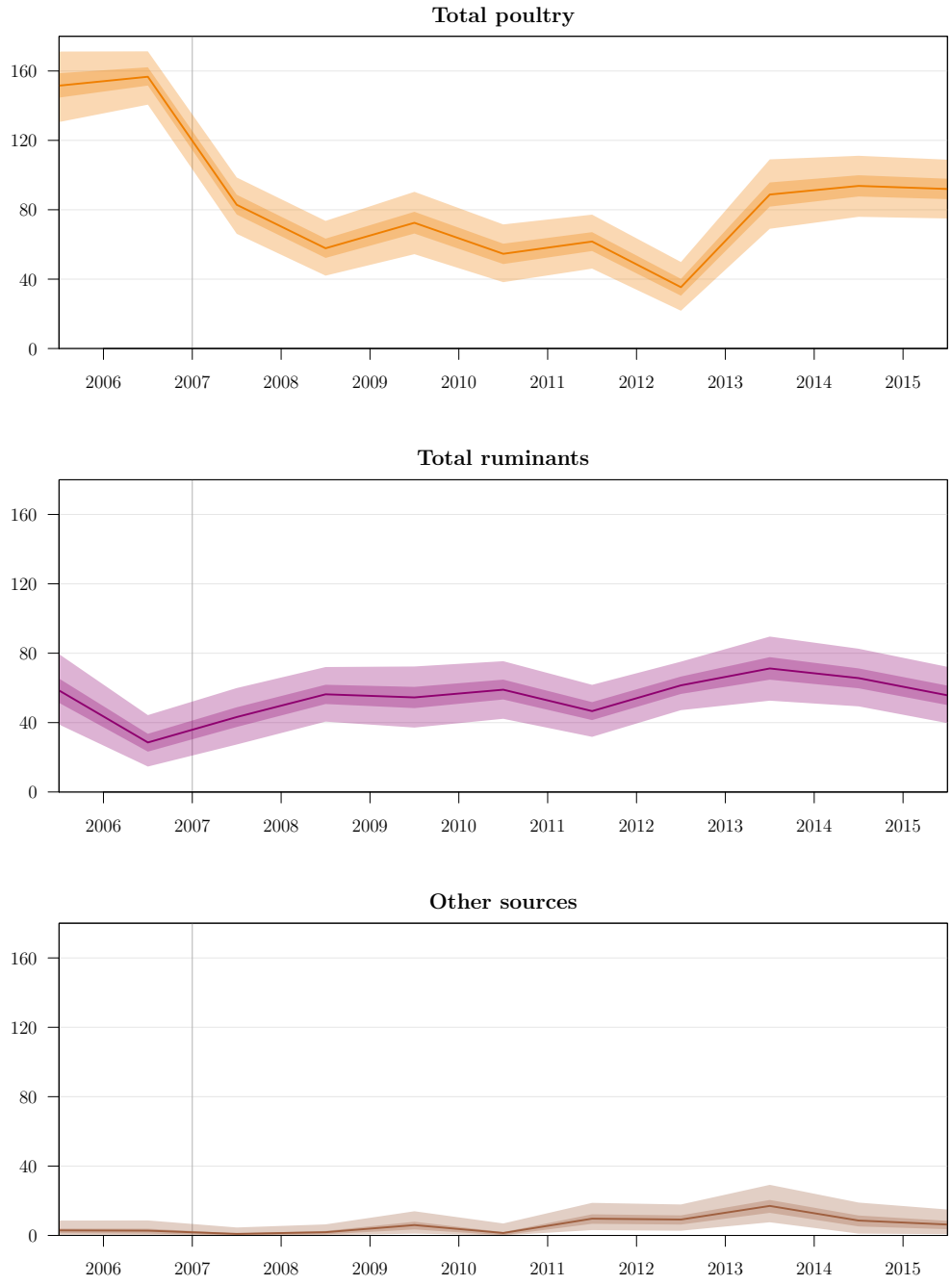

Figure 16: Estimated cases attributed to poultry, ruminant and other sources in the Manawatu between July 2005 and June 2016. Years 2006 and 2007 represent the pre-intervention period. Note that July to June is used to ensure the summer peak is centered within each period

Figure 17: Poultry, ruminant and other source attribution estimates for human cases in the Manawatu between July 2005 and June 2016. 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	126.7 (100.4, 150.9)	112.7 (84.5, 134.4)	64.1 (43.0, 83.1)	46.8 (26.2, 64.4)	60.2 (37.5, 80.2)	13.6 (0.0, 38.5)
Supplier B	21.5 (0.8, 39.3)	41.8 (20.6, 62.3)	11.8 (0.0, 27.5)	10.7 (0.1, 25.1)	8.0 (0.0, 22.6)	6.9 (0.0, 23.7)
Supplier Other	8.2 (0.0, 27.0)	3.0 (0.0, 17.0)	2.3 (0.0, 15.5)	1.4 (0.0, 8.5)	2.2 (0.0, 13.2)	30.1 (0.3, 59.7)
Duck	0.1 (0.0, 1.0)	0.1 (0.0, 0.7)	0.1 (0.0, 0.8)	0.1 (0.0, 0.5)	0.1 (0.0, 0.7)	0.1 (0.0, 1.0)
Turkey	0.3 (0.0, 3.0)	0.2 (0.0, 2.2)	0.2 (0.0, 2.2)	0.1 (0.0, 1.2)	0.2 (0.0, 2.3)	0.4 (0.0, 4.3)
Spent Hen	0.5 (0.0, 5.5)	0.9 (0.0, 9.6)	0.4 (0.0, 4.2)	0.2 (0.0, 2.3)	0.6 (0.0, 6.3)	0.7 (0.0, 7.7)
Cattle	48.6 (21.2, 72.8)	20.9 (0.7, 41.6)	44.4 (22.0, 63.6)	45.0 (15.9, 69.7)	54.1 (32.0, 74.3)	38.2 (0.7, 75.5)
Sheep	4.3 (0.0, 25.4)	4.5 (0.0, 21.4)	2.7 (0.0, 19.9)	9.4 (0.0, 36.0)	2.6 (0.0, 18.8)	22.9 (0.0, 60.0)
Dog/Cat	0.4 (0.0, 4.0)	1.3 (0.0, 13.8)	0.3 (0.0, 2.7)	0.3 (0.0, 3.3)	1.0 (0.0, 10.4)	1.0 (0.0, 11.6)
Wild Water Bird	0.5 (0.0, 2.3)	0.3 (0.0, 1.6)	0.2 (0.0, 0.8)	0.2 (0.0, 1.2)	0.5 (0.0, 2.3)	0.3 (0.0, 1.4)
Other Wild Bird	1.7 (0.0, 6.7)	2.3 (0.0, 8.0)	0.4 (0.0, 2.4)	1.7 (0.0, 6.1)	3.0 (0.1, 9.5)	0.8 (0.0, 4.8)
Water	0.2 (0.0, 1.5)	0.1 (0.0, 1.3)	0.1 (0.0, 1.5)	0.1 (0.0, 1.1)	0.5 (0.0, 4.8)	0.2 (0.0, 1.8)
	2011–2012	**2012–2013**	**2013–2014**	**2014–2015**	**2015–2016**	
Supplier A	16.1 (0.2, 35.9)	13.6 (0.1, 32.0)	21.7 (0.1, 52.3)	56.6 (29.6, 81.1)	31.9 (0.3, 62.3)	
Supplier B	21.5 (0.5, 40.2)	9.6 (0.0, 25.3)	26.9 (0.2, 52.3)	10.3 (0.0, 29.9)	39.6 (1.5, 65.2)	
Supplier Other	19.5 (0.2, 50.3)	7.1 (0.0, 25.4)	34.0 (1.9, 75.7)	21.8 (0.5, 50.6)	17.3 (0.0, 69.5)	
Duck	0.1 (0.0, 1.0)	0.1 (0.0, 0.7)	0.1 (0.0, 0.9)	0.1 (0.0, 1.5)	0.4 (0.0, 2.9)	
Turkey	0.4 (0.0, 4.0)	0.2 (0.0, 2.6)	0.2 (0.0, 2.0)	0.7 (0.0, 6.5)	0.7 (0.0, 6.7)	
Spent Hen	0.9 (0.0, 11.0)	0.6 (0.0, 6.5)	0.7 (0.0, 6.8)	1.3 (0.0, 15.3)	1.7 (0.0, 16.0)	
Cattle	46.7 (23.7, 65.4)	59.6 (34.8, 78.5)	58.2 (14.6, 91.7)	69.7 (51.3, 88.8)	35.8 (1.7, 70.8)	
Sheep	3.2 (0.0, 21.4)	5.6 (0.0, 28.8)	16.8 (0.0, 58.5)	1.5 (0.0, 10.4)	22.6 (0.0, 58.5)	
Dog/Cat	1.0 (0.0, 10.3)	0.5 (0.0, 4.9)	1.3 (0.0, 13.8)	0.6 (0.0, 6.4)	1.1 (0.0, 11.7)	
Wild Water Bird	0.6 (0.0, 3.5)	0.3 (0.0, 1.6)	0.7 (0.0, 3.2)	0.5 (0.0, 2.8)	0.5 (0.0, 2.3)	
Other Wild Bird	7.3 (0.1, 17.1)	8.6 (2.1, 18.1)	16.2 (6.5, 28.9)	4.8 (0.0, 15.5)	1.4 (0.0, 8.6)	
Water	0.7 (0.0, 6.6)	0.3 (0.0, 3.6)	0.2 (0.0, 2.3)	0.2 (0.0, 2.2)	0.9 (0.0, 7.3)	

Table 12: The estimated number of human cases of campylobacteriosis in the Manawatu attributable to each source for each year from July 2005– June 2016. 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.

	2005–2006	**2006–2007**	**2007–2008**	**2008–2009**	**2009–2010**	**2010–2011**
Supplier A	59.5 (47.1, 70.8)	60.0 (44.9, 71.5)	50.5 (33.9, 65.4)	40.3 (22.6, 55.5)	45.2 (28.2, 60.3)	11.9 (0.0, 33.4)
Supplier B	10.1 (0.4, 18.5)	22.2 (10.9, 33.2)	9.3 (0.0, 21.7)	9.3 (0.0, 21.7)	6.0 (0.0, 17.0)	6.0 (0.0, 20.6)
Supplier Other	3.8 (0.0, 12.7)	1.6 (0.0, 9.0)	1.8 (0.0, 12.2)	1.2 (0.0, 7.3)	1.6 (0.0, 10.0)	26.1 (0.3, 51.9)
Duck	0.1 (0.0, 0.5)	0.0 (0.0, 0.4)	0.1 (0.0, 0.6)	0.0 (0.0, 0.4)	0.1 (0.0, 0.5)	0.1 (0.0, 0.8)
Turkey	0.1 (0.0, 1.4)	0.1 (0.0, 1.2)	0.2 (0.0, 1.8)	0.1 (0.0, 1.0)	0.2 (0.0, 1.8)	0.3 (0.0, 3.7)
Spent Hen	0.2 (0.0, 2.6)	0.5 (0.0, 5.1)	0.3 (0.0, 3.3)	0.2 (0.0, 2.0)	0.4 (0.0, 4.7)	0.6 (0.0, 6.7)
Cattle	22.8 (10.0, 34.2)	11.1 (0.4, 22.1)	35.0 (17.3, 50.1)	38.8 (13.7, 60.1)	40.7 (24.1, 55.9)	33.2 (0.6, 65.6)
Sheep	2.0 (0.0, 11.9)	2.4 (0.0, 11.4)	2.2 (0.0, 15.7)	8.1 (0.0, 31.0)	2.0 (0.0, 14.2)	19.9 (0.0, 52.2)
Dog/Cat	0.2 (0.0, 1.9)	0.7 (0.0, 7.3)	0.2 (0.0, 2.1)	0.3 (0.0, 2.8)	0.7 (0.0, 7.8)	0.8 (0.0, 10.0)
Wild Water Bird	0.2 (0.0, 1.1)	0.2 (0.0, 0.8)	0.1 (0.0, 0.7)	0.2 (0.0, 1.0)	0.3 (0.0, 1.8)	0.2 (0.0, 1.2)
Other Wild Bird	0.8 (0.0, 3.2)	1.2 (0.0, 4.2)	0.3 (0.0, 1.9)	1.4 (0.0, 5.3)	2.3 (0.1, 7.1)	0.7 (0.0, 4.2)
Water	0.1 (0.0, 0.7)	0.1 (0.0, 0.7)	0.1 (0.0, 1.2)	0.1 (0.0, 1.0)	0.4 (0.0, 3.6)	0.2 (0.0, 1.6)
	2011–2012	**2012–2013**	**2013–2014**	**2014–2015**	**2015–2016**	
Supplier A	13.6 (0.1, 30.4)	12.8 (0.1, 30.2)	12.3 (0.0, 29.5)	33.7 (17.6, 48.2)	20.7 (0.2, 40.4)	
Supplier B	18.3 (0.4, 34.1)	9.0 (0.0, 23.9)	15.2 (0.1, 29.6)	6.1 (0.0, 17.8)	25.7 (1.0, 42.4)	
Supplier Other	16.5 (0.1, 42.6)	6.7 (0.0, 24.0)	19.2 (1.1, 42.7)	13.0 (0.3, 30.1)	11.2 (0.0, 45.1)	
Duck	0.1 (0.0, 0.9)	0.1 (0.0, 0.6)	0.1 (0.0, 0.5)	0.1 (0.0, 0.9)	0.2 (0.0, 1.9)	
Turkey	0.3 (0.0, 3.4)	0.2 (0.0, 2.5)	0.1 (0.0, 1.1)	0.4 (0.0, 3.9)	0.5 (0.0, 4.4)	
Spent Hen	0.8 (0.0, 9.3)	0.6 (0.0, 6.1)	0.4 (0.0, 3.8)	0.8 (0.0, 9.1)	1.1 (0.0, 10.4)	
Cattle	39.5 (20.1, 55.4)	56.2 (32.8, 74.1)	32.9 (8.2, 51.8)	41.5 (30.5, 52.8)	23.3 (1.1, 46.0)	
Sheep	2.7 (0.0, 18.2)	5.3 (0.0, 27.1)	9.5 (0.0, 33.0)	0.9 (0.0, 6.2)	14.7 (0.0, 38.0)	
Dog/Cat	0.8 (0.0, 8.7)	0.4 (0.0, 4.6)	0.7 (0.0, 7.8)	0.4 (0.0, 3.8)	0.7 (0.0, 7.6)	
Wild Water Bird	0.5 (0.0, 2.9)	0.3 (0.0, 1.5)	0.4 (0.0, 1.8)	0.3 (0.0, 1.7)	0.3 (0.0, 1.5)	
Other Wild Bird	6.2 (0.1, 14.5)	8.1 (1.9, 17.1)	9.1 (3.7, 16.3)	2.8 (0.0, 9.2)	0.9 (0.0, 5.6)	
Water	0.6 (0.0, 5.6)	0.3 (0.0, 3.4)	0.1 (0.0, 1.3)	0.1 (0.0, 1.3)	0.6 (0.0, 4.7)	

Table 13: The estimated percentage of human cases of campylobacteriosis in the Manawatu attributable to each source for each year from July 2005– June 2016. 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 18 shows the output from the dynamic Island model, displaying the attribution to poultry, ruminant, water, and other sources over the 12 year period. In 2016 there were significantly more cases than in 2015, being most similar to 2014. Figure 19 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 2016, the profiles are a little different, with ruminant cases peaking a little earlier than the poultry cases towards the end of 2016.

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 20).

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 in 2016 the increase is mostly in rural areas rather than urban areas.

Interestingly, in 2016 the difference in attribution between rural and urban dwellers is less clear than was seen in 2015, more similar to what is seen in 2014 (Figures 21 and 22). We might expect things to change year on year in this measure, as much is dominated by the summer peak that tends to be urban and poultry associated, and this shifts from year to year. Nonetheless, this year the increase in November/December was mostly a rural effect, so things may be a little different, with a comparative increase in poultry

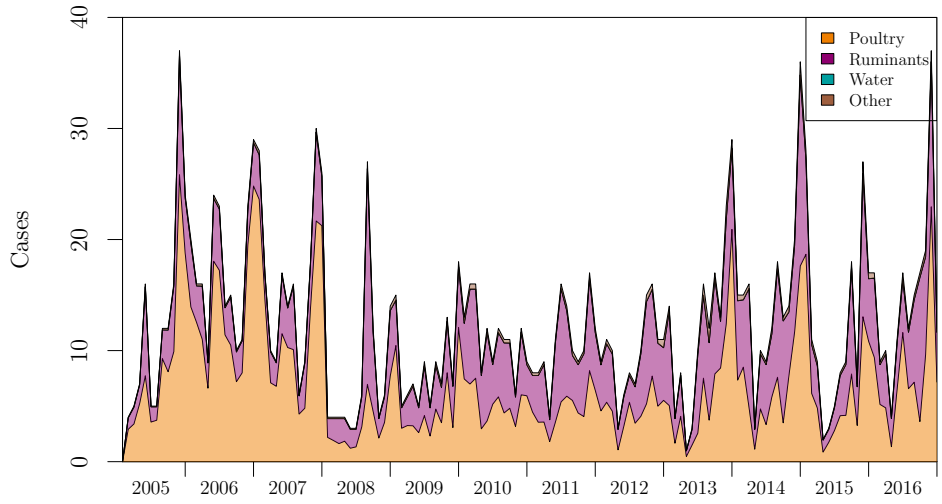

Figure 18: Estimated number of human cases per month attributed to each source by the dynamic Island model from 2005 to 2016. Colours indicate the source the cases are attributed to: poultry (orange), ruminants (purple), water/environmental (blue) and other (brown).

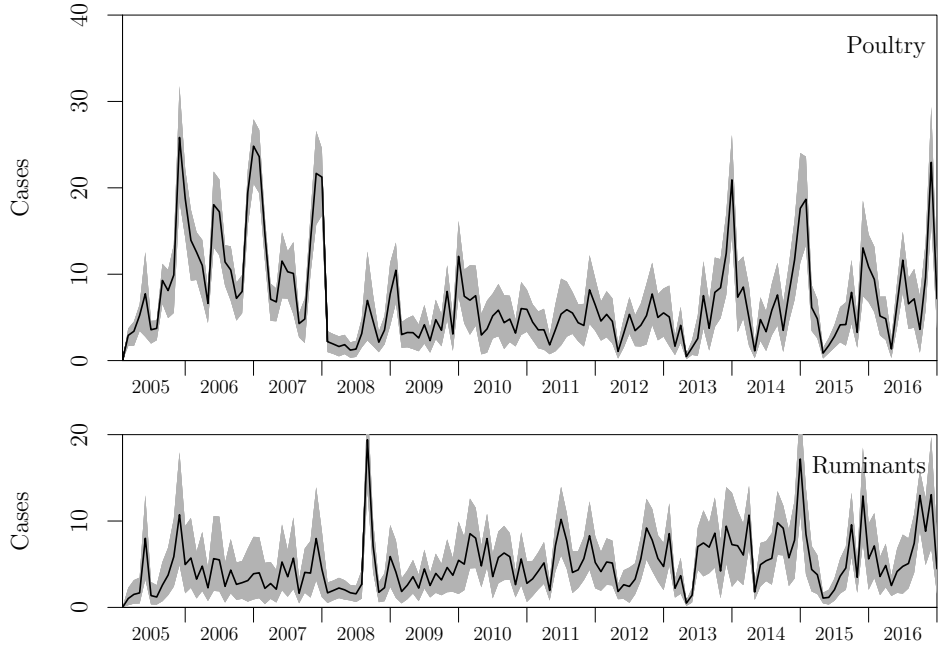

Figure 19: Estimated number of human cases per month attributed to poultry and ruminants by the dynamic Island model with 95% credible intervals from 2005 – 2016.

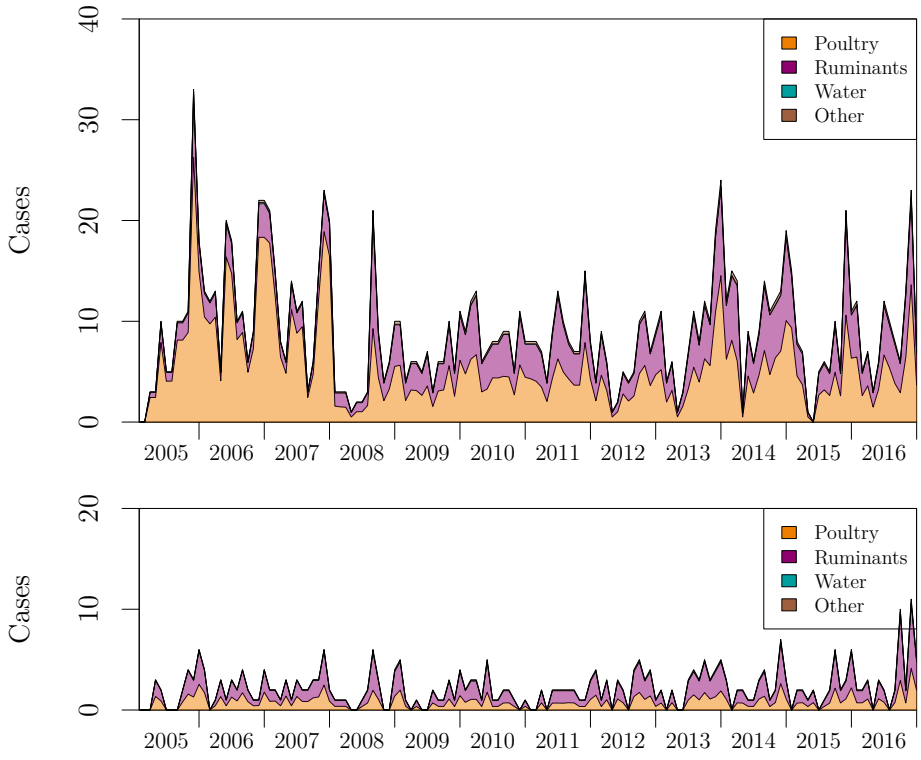

Figure 20: Urban and rural cases 2005–2016: Estimated number of human cases attributed to each reservoir per month by the dynamic Island model.

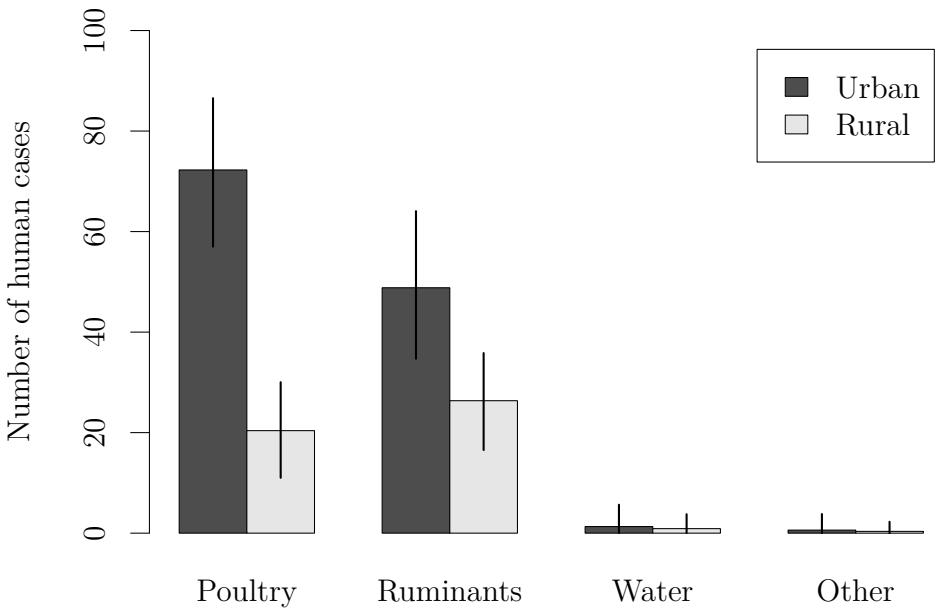

Figure 21: Urban and Rural cases 2016: Estimated number of human cases attributed to each reservoir determined by the Island model.

attributed rural cases.

In addition to the asymmetric island model, we also assessed urban and rural attribution before and after the intervention in the poultry industry using `sourceR`. Figures 23 and 24 shows the attribution to the three main source groups (total poultry, total ruminants, other) for urban and rural dwellers for 2005–2007 and 2008–2016. 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.

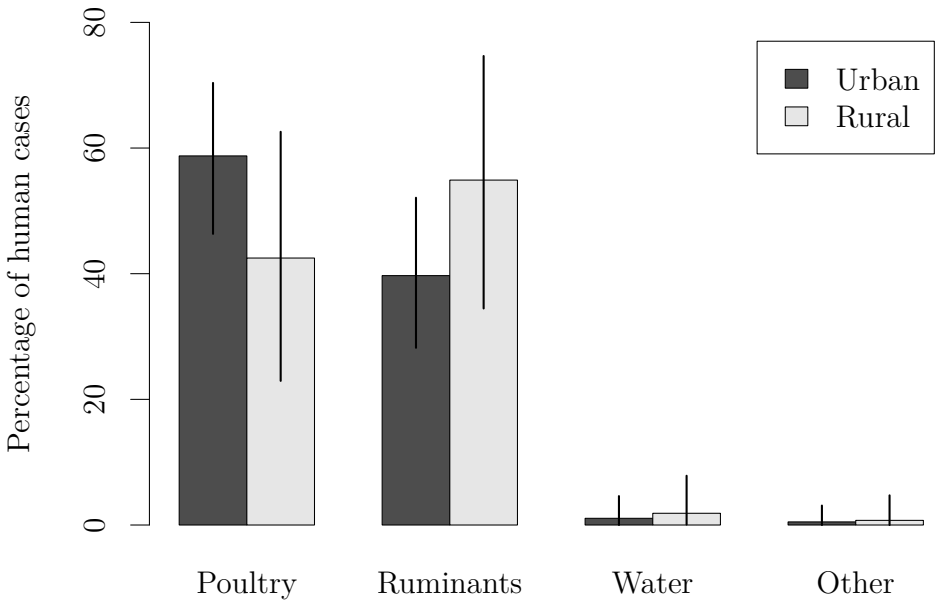

Figure 22: Urban and Rural cases 2016: Estimated proportion of human cases attributed to each reservoir determined by the Island model.

Figure 25 presents the types with largest q-values. Interestingly the top sequence type is still ST-474 even though it is no longer highly prevalent among human cases, which shows how dominant this strain was in 2005–2007. Both *C. jejuni* and *C. coli* types feature, as does the new poultry-related strain ST-6964.

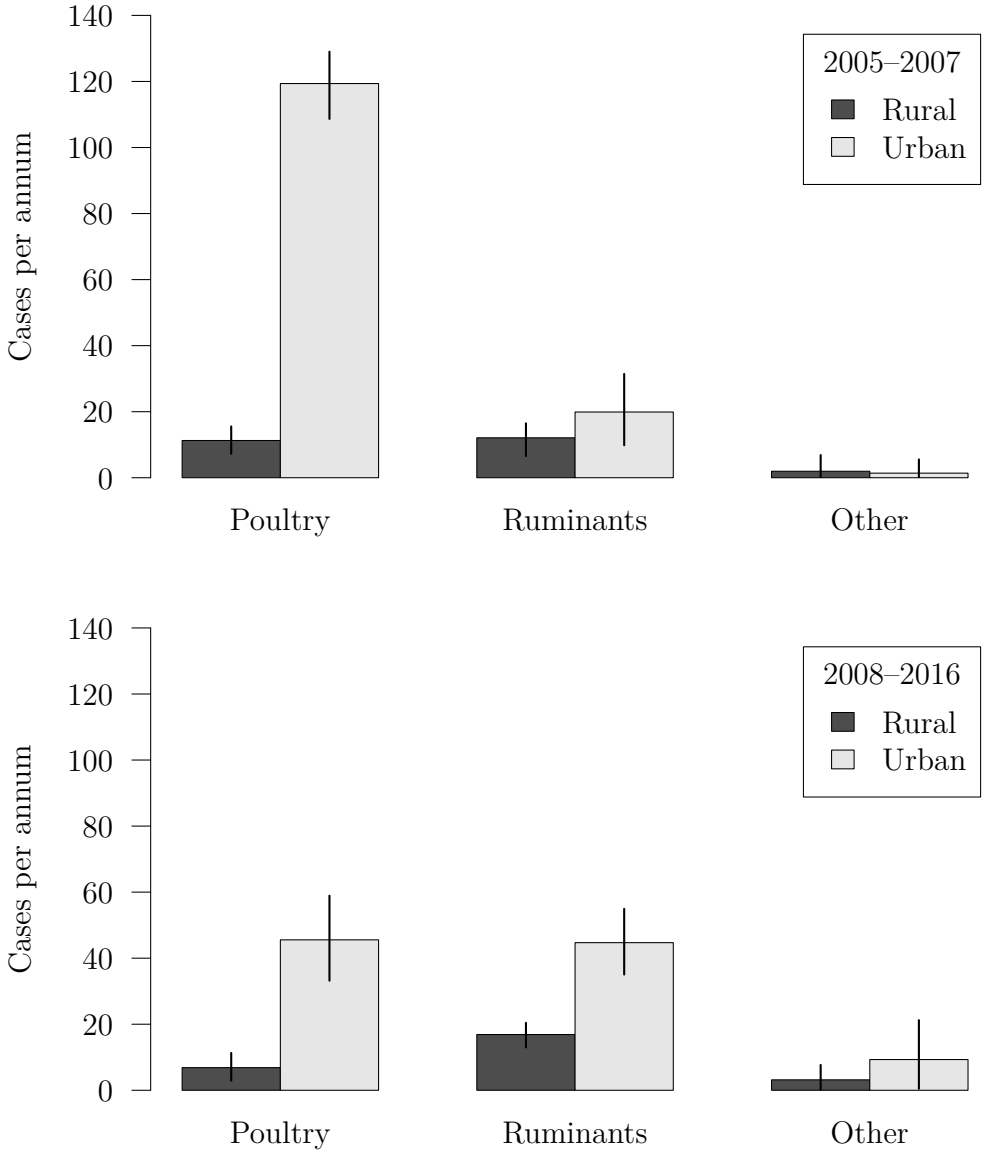

Figure 23: Estimated number of urban and rural cases per annum attributed to each reservoir 2005–2007 (top) and 2008–2016 (bottom) as determined using `sourceR`.

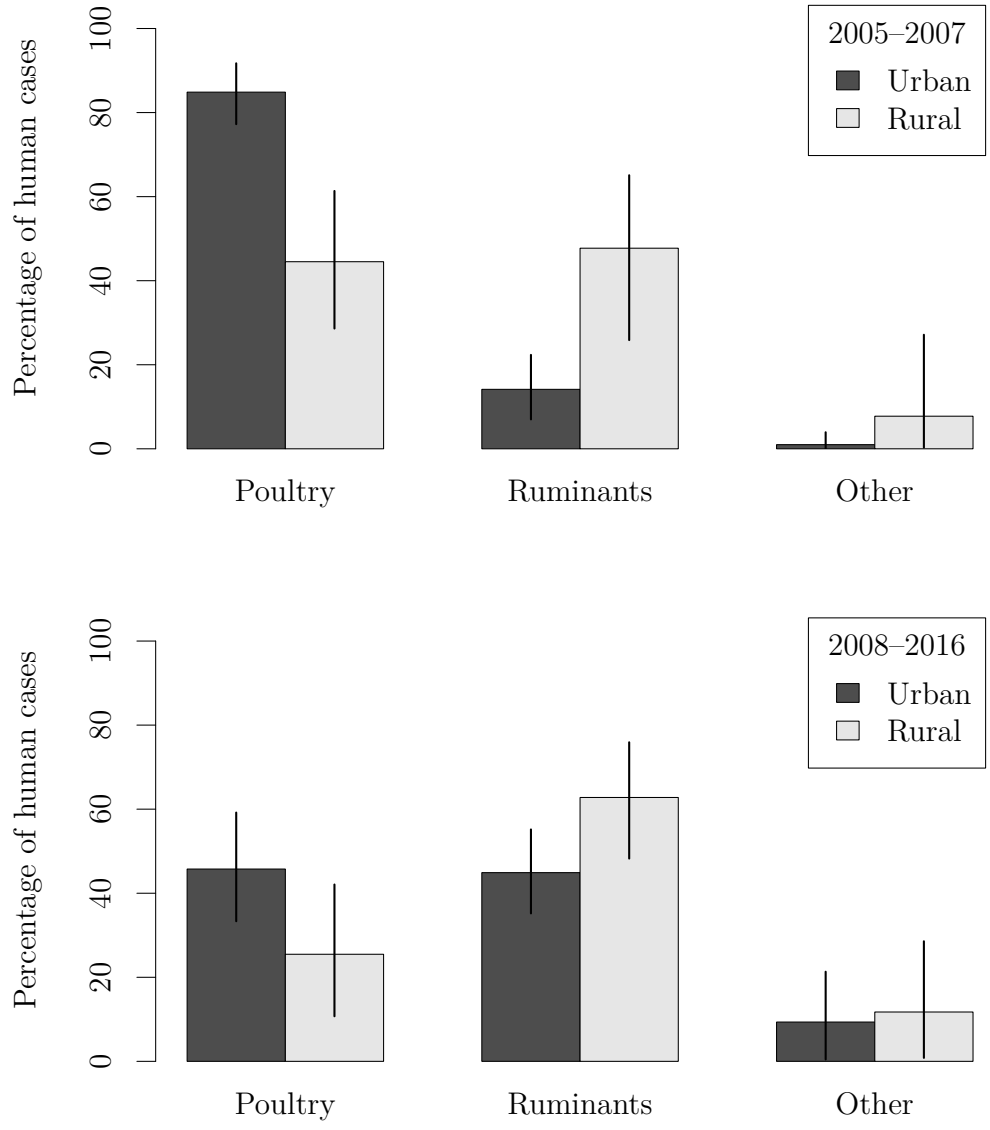

Figure 24: Estimated proportion of urban and rural cases attributed to each reservoir 2005–2007 (top) and 2008–2016 (bottom) as determined using `sourceR`.

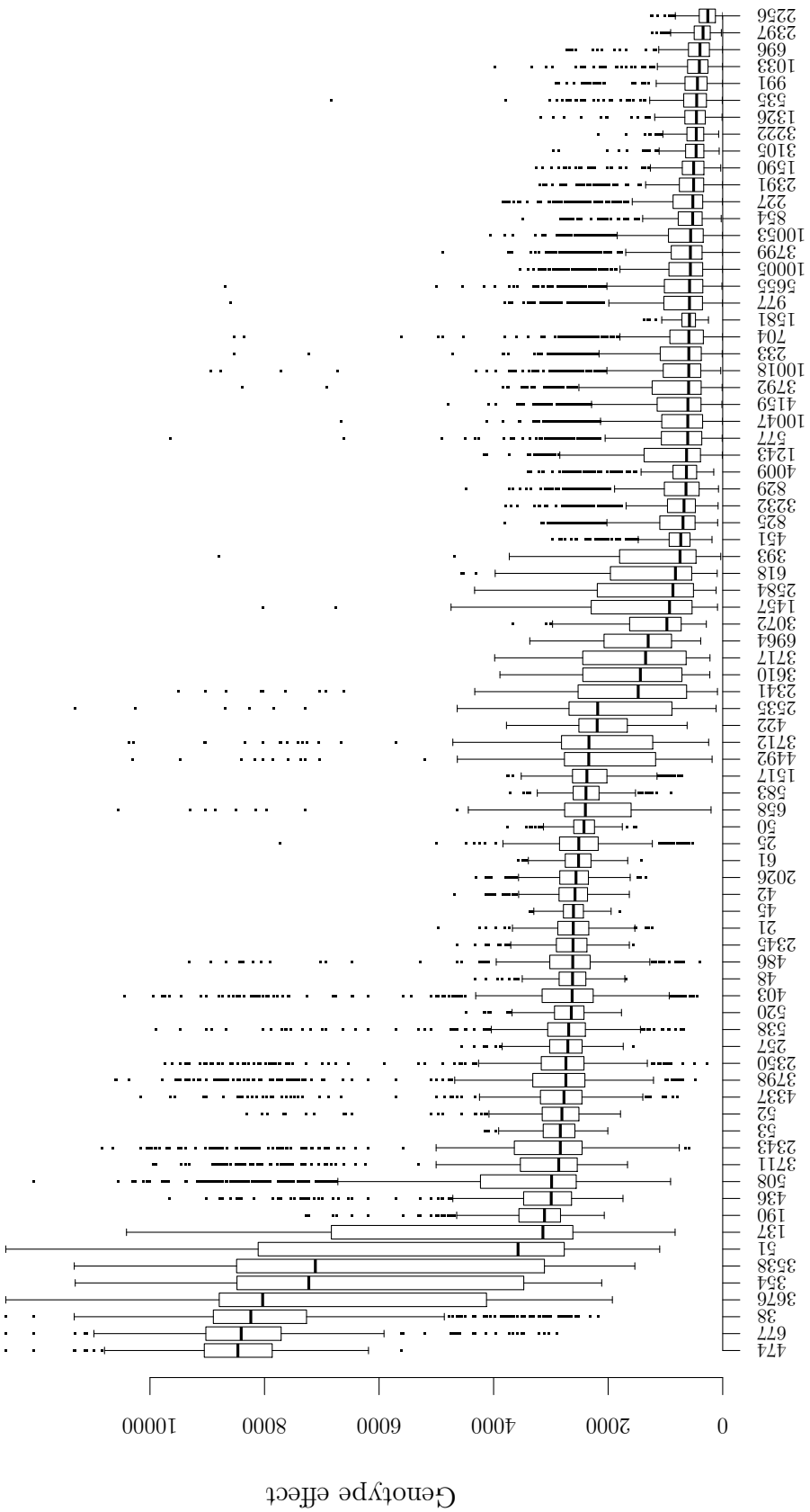

Figure 25: Genotype effects from the `sourceR` model, with larger values indicating that the sequence type is overrepresented among human cases compared to other types.

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 for 2012 through 2016 are shown in Figure 26 and Figure 27. Of interest is whether the large outbreak in Hawke's Bay in August 2016 due to water supply contamination (excluded in the figures) has given rise to increasing case rates due to higher reporting following this highly publicised event.

We see an increase in the case rates in MidCentral, particularly towards the end of the 2016, which is also shown in the neighbouring regions of Whanganui and Wairarapa. Other regions, including Hawke's Bay, however, don't show this same increase. It might be that these localised changes are driven by increased reporting rather than by an increasing case rate. However, the lack of a substantial increase in Hawke's Bay and other areas suggests that it is unlikely to be solely a reporting artifact.

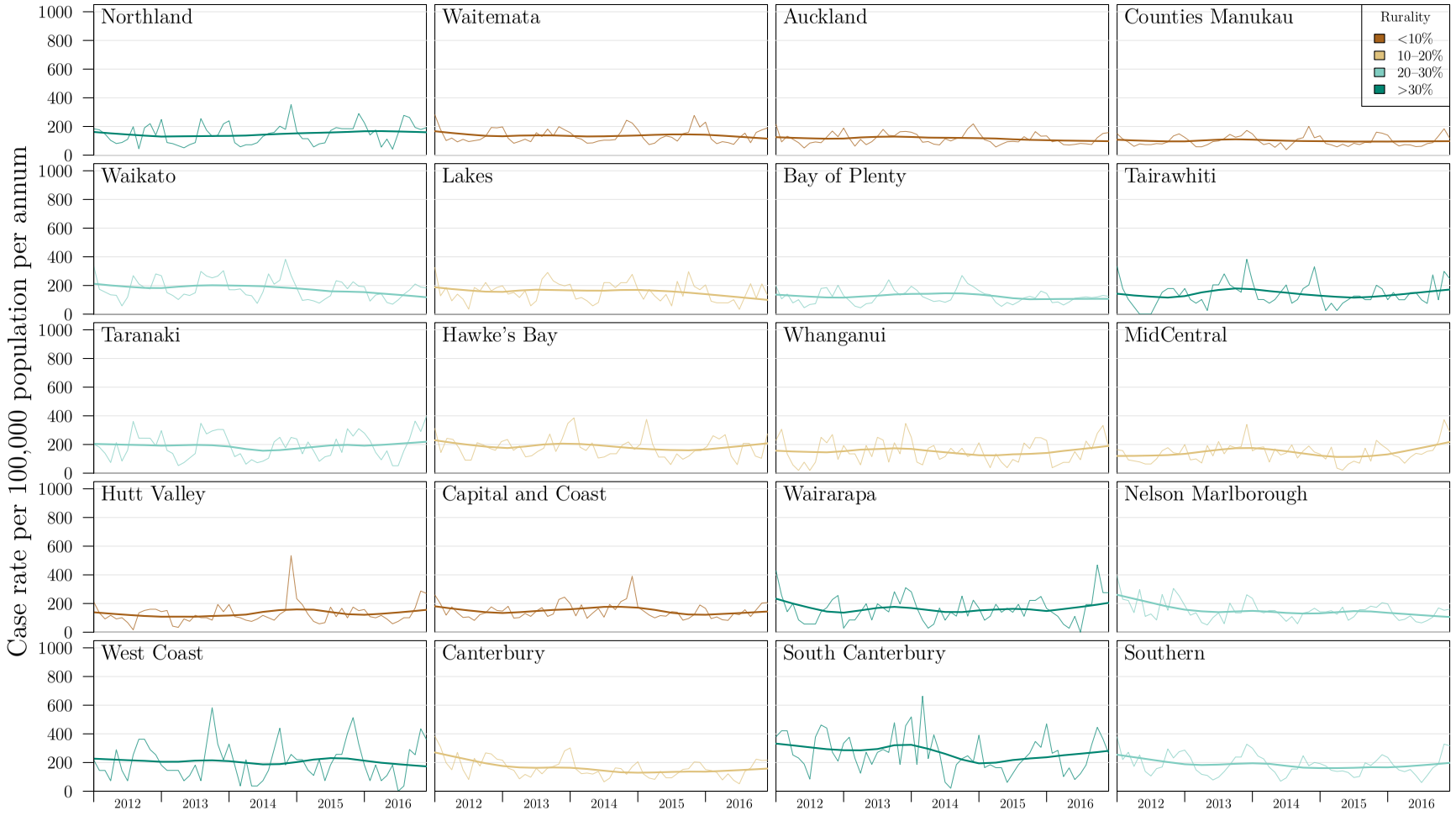

Figure 26: Comparison of trends in case rates per 100,000 population across district health boards. The Havelock North outbreak cases have been excluded. Light lines are the data, and heavy curves represent smooth trends after accounting for seasonality (Figure 27). Colours represent the percentage of rural population per DHB.

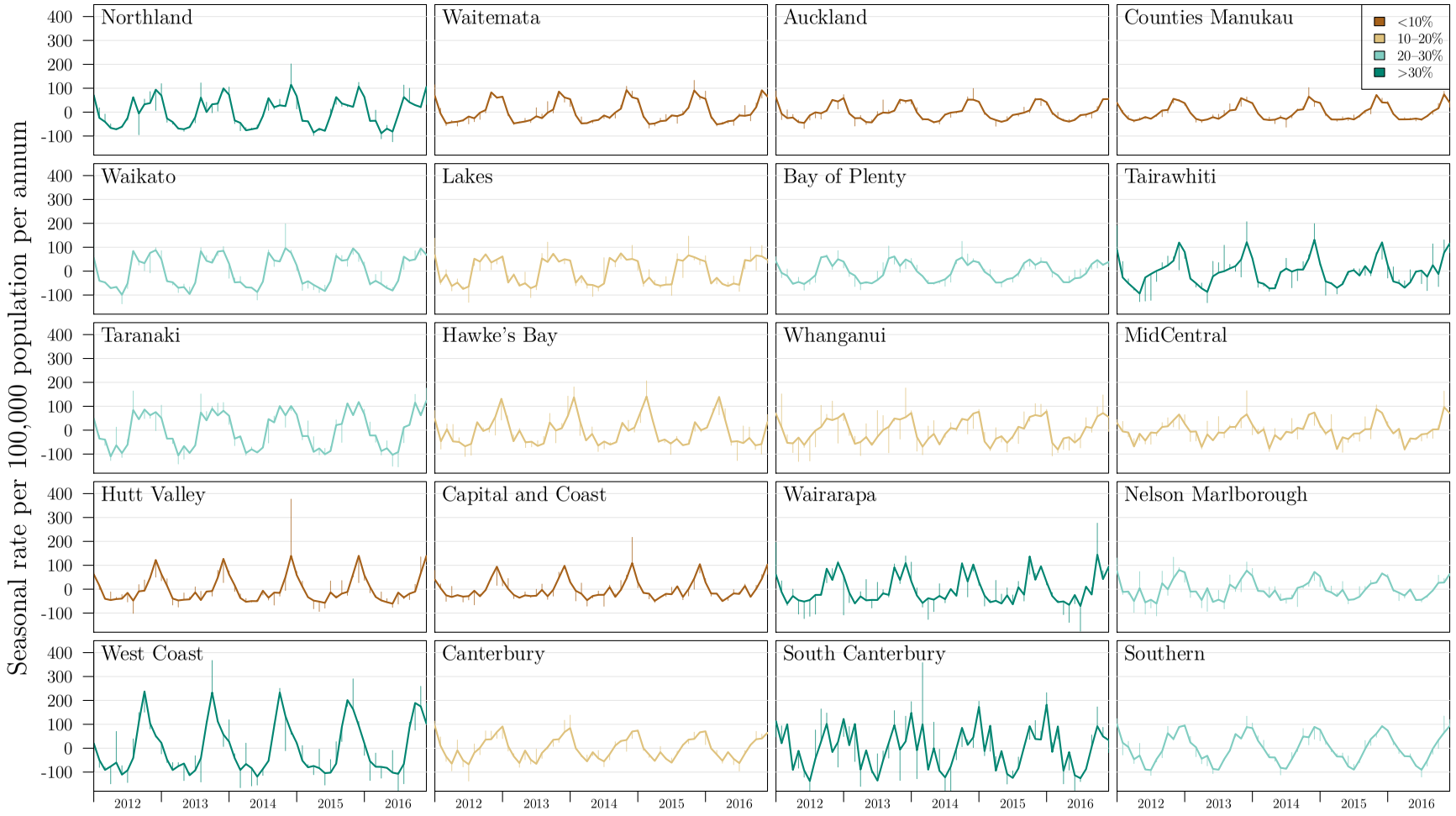

Figure 27: Comparison of seasonality in case rates per 100,000 population across district health boards. The Havelock North outbreak cases have been excluded. Vertical lines represent residuals after smooth trends (Figure 26) and seasonality (heavy curves). Colours represent the percentage of rural population per DHB

This is confirmed in comparing the trend for MidCentral DHB (Figure 28) in 2016 compared with NZ as a whole (again, the Havelock North outbreak cases have been removed). We note that the bump in MidCentral trend during 2013 might 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. There was an additional change in February 2016, with MedLab Central now doing culturing which was shown to be more specific again, so it might be that some of the increase seen in 2016 may be partly due to fewer false negatives.

To further characterise this increase, the cases for MidCentral were divided into urban and rural subgroups and trends through time were compared (Figure 29). The marked increase in 2016 can be seen to be a predominantly rural effect, particularly in November and December. This increase dominates in the seasonal trends which have become more marked over time in both urban and rural areas. We note that this may still be consistent with increased reporting rather than an increase in cases, as rural people are more likely to have water supplies susceptible to contamination, and thus be more likely to report cases following news of the water-borne outbreak in Havelock North.

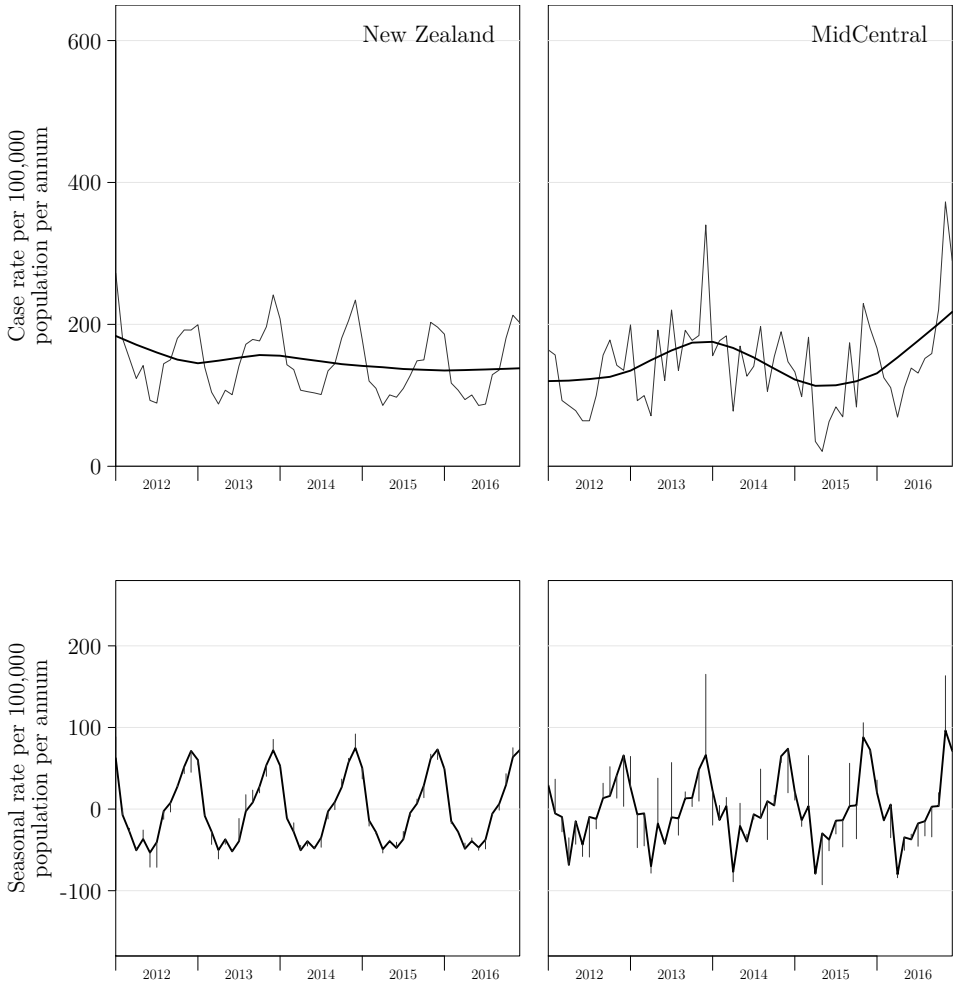

Figure 28: New Zealand (left, Havelock North outbreak cases excluded) and Midcentral DHB (right) campylobacteriosis case rates over time. Rates and smooth trend (top) and seasonality with residuals (bottom).

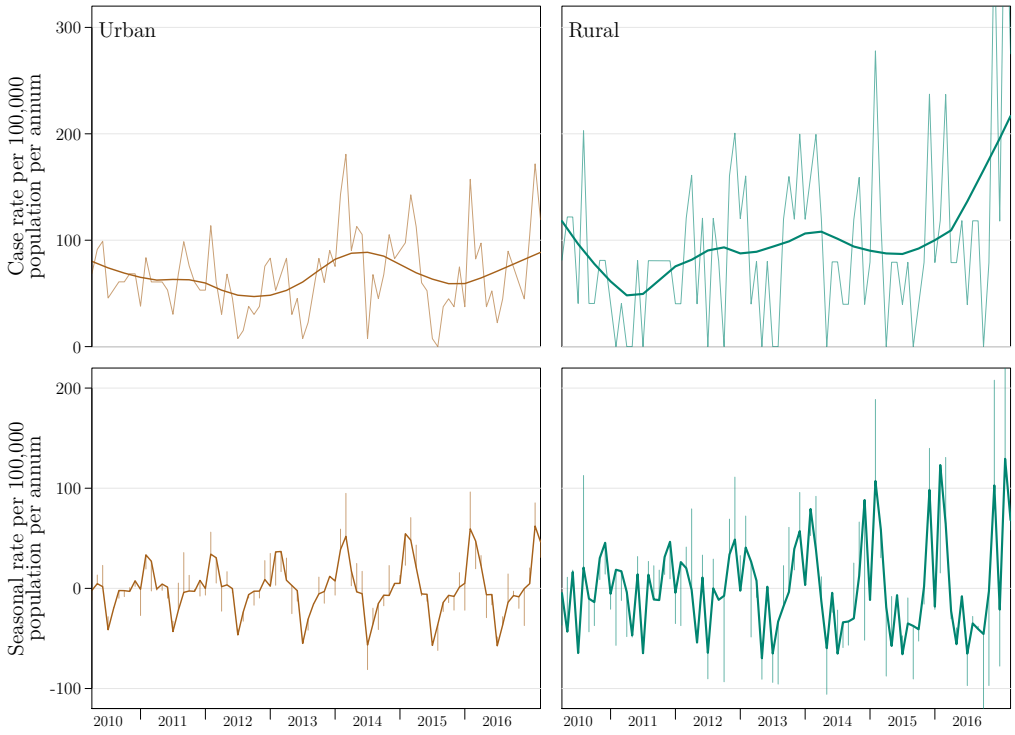

Figure 29: Case rates over time for urban (left) and rural (right) populations within the study region. Rates and smooth trend (top) and seasonality with residuals (bottom).

5 Discussion and Conclusions

This report provides an update of the molecular epidemiology of campylobacteriosis in the Manawatu in 2016 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.

The attribution of urban and rural cases in 2016 shows slightly more urban cases were attributed to poultry compared with rural cases which were more likely attributed to ruminant sources. As noted in previous years, attribution within rural dwellers from month to month is typically more stable than within urban dwellers, with most of the variability in urban cases being in the poultry attributed cases which tend to peak more sharply in the summer months.

There has been an increased proportion of *C. coli* isolates from poultry since 2013 compared to previous years. It was initially thought that this may in part be due to a more rigorous investigation of non-*jejuni* isolates using alternate methods, 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'. A comparison of the two methods used, however, showed that the alternate method resulted in a smaller increase in *C. coli* prevalence than the main method which has been in use since 2005. Further, we note that *C. coli* STs have been found in the latest round of ruminant sampling as well.

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 one of the dominant types found from poultry suppliers A through C, accounting for 14% of all isolates, and has also been found in supplier D this year. The type has also been seen in a further 14 human cases, while not being found in other non-poultry sources.

Preliminary work on the use of whole genome MLST looking at the attribution of ST-474 showed that ST-474s observed both among humans and on the primary source (supplier A) were different before and after the poul-

try intervention of 2008. It is currently unknown whether other types show similar temporal variation, or variation among sources when looked at using whole genome sequence data, but it is clear that there is potential to utilise the additional level of detail that whole genome sequencing provides in informing source attribution.

With 3 rounds of ruminant sampling being completed as of the end of 2016, we have continued to identify sequence types that were not seen in the last round of sampling in 2005–2008, including sequence types that have yet to be designated an ST number in the PubMLST database suggesting they have not been previously identified on any source. Many of these types have been also seen in poultry and among human cases, and thus identifying that these types are also found among ruminants is important for the attribution of individual human cases.

There was a marked increase in notified cases within the Manawatu region in 2016, particularly in the later months. This may in part be due to the change in methodology adopted by MedLab central, which is moved from ELISA testing to confirmation by culture, where the latter is considered more sensitive. This corresponds with an increase in the proportion of swabs that we can confirm are positive in the Hopkirk Laboratory.

An analysis of all samples submitted to MedLab central between 2014 and 2015 showed that of the 671 samples that were not submitted to mEpiLab, 29 (4.3% were found to be positive for *Campylobacter*. A comparison of the STs from false negatives and true positives using permutational analysis of variance showed little evidence (P=) for a difference in ST distribution, so that there is likely to be minimal impact on the source attribution models due to these missing samples. As the culture method is now more sensitive, it would be expected that there would be fewer false negatives in 2016 compared to when this study was done.

In addition, the increase in notifications might be a consequence of higher reporting following the much publicised waterborne outbreak of campylobacteriosis in Havelock North in August 2016, with people being more mindful of the risks of *Campylobacter* infection and thus more likely to report. A comparison of the case rates of district health boards through time, however, showed that this increase in 2016 was mostly confined to the Manawatu

region in addition to neighbouring regions of Whanganui and Wairarapa. Importantly, this increase was not seen across New Zealand as a whole, or in the Hawke's Bay region once the Havelock North outbreak cases were removed. In addition, the increase in notifications within the Manawatu region was confined to rural areas, with urban case rates remaining similar. Thus, it is unlikely that the increased notifications is solely an artifact of increased reporting, and may indicate an increased exposure in the rural community.

It would be interesting to assess the trends in rural case rates in 2016 and earlier among the other DHBs, however this would require case numbers by rurality over time which are not available through public data releases.

6 Acknowledgments

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

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