MASSEY UNIVERSITY
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Report: NZFSA Agreement 11777, Schedule 1A Source attribution July 2009 to June 2010 of human *Campylobacter jejuni* cases from the Manawatu

December 2010

prepared for
the New Zealand Food Safety Authority

by
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Report: 11424

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1 Summary

This report describes the results of multilocus sequence typing and source attribution modelling of isolates stored in the culture bank of *Campylobacter jejuni* samples from poultry and humans in the Manawatu sentinel site. These were cataloged and stored in the Hopkirk mEpiLab between July 2009 and June 2010. The marked decline in human cases attributable to poultry after the introduction of the Campylobacter in Poultry Risk Management Strategy in 2006, is still evident in 2009/10. In the pre-intervention period over 70% of human cases were attributable to poultry, whereas in the post-intervention years 2008 through to 2010 this estimate has declined to less than 50%. The most marked change was observed for poultry supplier A, whose attribution estimates declined from over 60% pre-intervention to less than 40% post-intervention. The proportion of cases attributable to genotype ST 474, which has been consistently associated with poultry supplier A, declined from approximately 40% in the pre-intervention period to approximately 20% in the post intervention period, and accounted for 19.4% in 2009/10. However, despite the relative increase in the contribution of ruminants to human cases, poultry still remains the most important source of human infection in the Manawatu. Recent trends in human cases indicate that the rate reduction in Central and Lower North Island, which includes the Manawatu, is lower than elsewhere in New Zealand. This may indicate that the decline in poultry associated cases in the Manawatu, although marked, may be less than that observed in other regions of New Zealand.

2 Introduction

NZFSA has 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 achieving this organisational goal. However 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.

The NZFSA wished to establish a bank of appropriate *Camplobacter 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 120 samples of human isolates (that have been collected and stored as part of agreement number 11424 between NZFSA and the Contractor) for multilocus sequence typing:

2. Select 80 samples of poultry carcase isolates (that have been collected and stored as part of the agreement number 11424 between NZFSA and the Contractor) for multilocus sequence typing:

3. Use the sequence typing to populate dynamic source attribution models developed by the Contractor as part of agreement number 11178 between the Contractor and NZFSA and detailed in the final report in Milestone 2 of Schedule 1 of that Agreement; and

4. Prepare and submit a draft report to NZFSA for comment detailing the outcomes of clause 3.3 of the schedule; and

5. Prepare and submit a final report to NZFSA's satisfaction detailing the outcomes of clause 3.3 of the schedule.

Details of the culture bank and microbiological investigations, including enumeration studies conducted between July 2009 and June 2010, were reported in the document: NZFSA Agreement 11424 Culture bank of *Campylobacter jejuni* samples from the Manawatu [3]. We recommend this report be read in conjunction with the above document.

3 Methods

3.1 Sampling and microbiology

These were outlined in detail elsewhere [3, 7].

3.1.1 Human faecal samples

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

3.1.2 Poultry carcases

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

Details of the enumeration of *Campylobacter* on poultry carcases are described elsewhere [3] and not covered in this report.

3.1.3 Multilocus sequence typing

MLST of *C. jejuni* isolates was performed using seven house-keeping genes: *asp*A (aspartase A), *gln*A (glutamine synthase), *glt*A (citrate synthase), *gly*A

(serine hydroxymethyltransferase), *pgm* (phosphoglucomutase), *tkt* (transketolase) and *unc*A (ATP synthase alpha subunit) based on the method outlined by Dingle et al., [1]. Alleles that did not give clear results were re-amplified and sequenced using primers sets published by Miller et al., (2005)[5]. Sequence data were collated by Dr Phil Carter at ESR, and alleles assigned using the Campylobacter PubMLST database (http://pubmlst.org/campylobacter).

3.2 Data analysis

3.2.1 Annual source attribution estimates

Source attribution estimates for the 12 months between 1st July 2009 and the 30th June 2010 were calculated using the assymetric island model as described elsewhere [8, 2, 7]. This was repeated for each of the preceding 4 years.

3.2.2 Dynamic source attribution modelling

Temporal variation was added to the modified Hald model [6], an existing Bayesian source attribution model. We model the observed number of isolates from each source at each time point by,

$$o_{jt} \sim \text{Poisson}\left(\sum_{i=0}^{T} \lambda_{ijt}\right),$$

where λ_{ijt} is the expected number of isolates of type i in source j at time t. In the same way as described in [4], we modelled λ_{ijt} by assuming that source-specific factors would vary more with time (particularly as a result of interventions) than type-specific factors and prevalence within the animal population, so that

$$\lambda_{ijt} = a_{jt} q_i p_{ij}.$$

The priors on the type-specific factors q_i and prevalence on the sources p_{ij} were log-normal and beta distributions as described in [6], and the priors for

the a_{jt} terms consist of an auto-regressive (AR1) process,

$$\log(a_{jt}) \sim N(\alpha_j + \theta \log(a_{j(t-1)}), \sigma).$$

The hyper-parameters were modelled using

$$\begin{aligned} \alpha_j &\sim N(0, 10), \\ \theta &\sim \text{Uniform}(0, 1) \\ \log(a_{j0}) &\sim N(0, 10), \\ \sigma &\sim \text{Gamma}(0.1, 0.1). \end{aligned}$$

These priors allow the source-specific factors such as controls in the food processing chain to change over time. Further model runs with alternate priors (e.g. using the independent priors of [4]) showed little dependence on the choice of priors.

4 Results

4.1 Distribution of MLST genotypes in humans and poultry

Table 1 shows the distribution of sequence types (STs) isolated from human cases in the Manawatu. The proportion of human cases with each ST in previous years is indicated. The proportion of cases attributable to ST 474 in 2009/10 was 19.4%, which is similar to the value in the previous 2 years, coinciding with the decline following the intervention in the poultry industry. Prior to the intervention, the proportion of cases attribtable to ST 474 was higher (31.1% and 38.7% in 2006/7 and 2005/6 respectively).

4.2 Source attribution estimates for human cases in 2009/10 compared to previous years

Figure 1 shows the source attribution estimates for the pre-intervention period 1st July 2005 to 30th June 2006 and Figure 2 shows the equivalent plot for the most recent 12 month period between 1st July 2009 and 30th June 2010. The marked decline in the proportion of cases attributable to poultry is evident, particularly for Supplier A. The increase in relative contribution from ruminants is also evident.

Figures 3 and 4 summarise all sources over the five-year period beginning on the 1st July 2005. The attribution estimates are summed in Figure 4 to show the estimated attribution to all chicken, all ruminants and other sources. The complete table of estimates, including credible intervals is shown in Table 2. With the exception of the trends in poultry and ruminants, these show reasonable consistency across all years.

Table 1: The distribution of multilocus sequence types in human cases in 2009/10 compared with the distribution in poultry supplies and human cases in the preceding 4 years.

		Poultry supplier			human isolates (%)				
ST	Human	A	B	C	2009/10	2008/9	2007/8	2006/7	2005/6
21	3				2.2	0.8	1.8	1.0	1.4
42	5	1			3.7	3.0	6.4	2.1	4.1
45	16		6		11.9	7.6	8.2	13.0	4.6
48	7		9		5.2	4.5	4.5	11.9	7.8
50	14		2		10.4	7.6	11.8	3.1	3.2
51	1				0.7				0.5
53	10	3	2		7.5	6.8	9.1	3.6	5.1
61	7		2		5.2	6.8	0.9	3.6	2.8
190	2				1.5	9.1	0.9	2.6	5.5
257	4				3.0	0.8	1.8	1.6	3.2
356		1			0.0				
403	2				1.5	1.5			0.5
436	2				1.5	4.5	0.9	1.6	0.5
474	26	10			19.4	22.0	19.1	31.1	38.7
520	2				1.5	0.8	0.9	0.5	1.4
535	1				0.7				
583	5				3.7	2.3	6.4	0.5	0.9
677	2				1.5	0.8	1.8	1.6	0.5
696	1				0.7				
1033	1				0.7				
1326	1	1			0.7				
1517	3			1	2.2		0.9		1.4
2026	3				2.2	0.8	3.6	0.5	3.7
2380	2				1.5				
2388	1				0.7				
2389		1			0.0				
3538	3				2.2		0.9		
3676	1				0.7	2.3	0.9	1.0	
3711	6		1		4.5		0.9	0.5	
3799	1				0.7				
4492	1				0.7				
NEW	2	1			1.5				
Total	134	18	22	1					

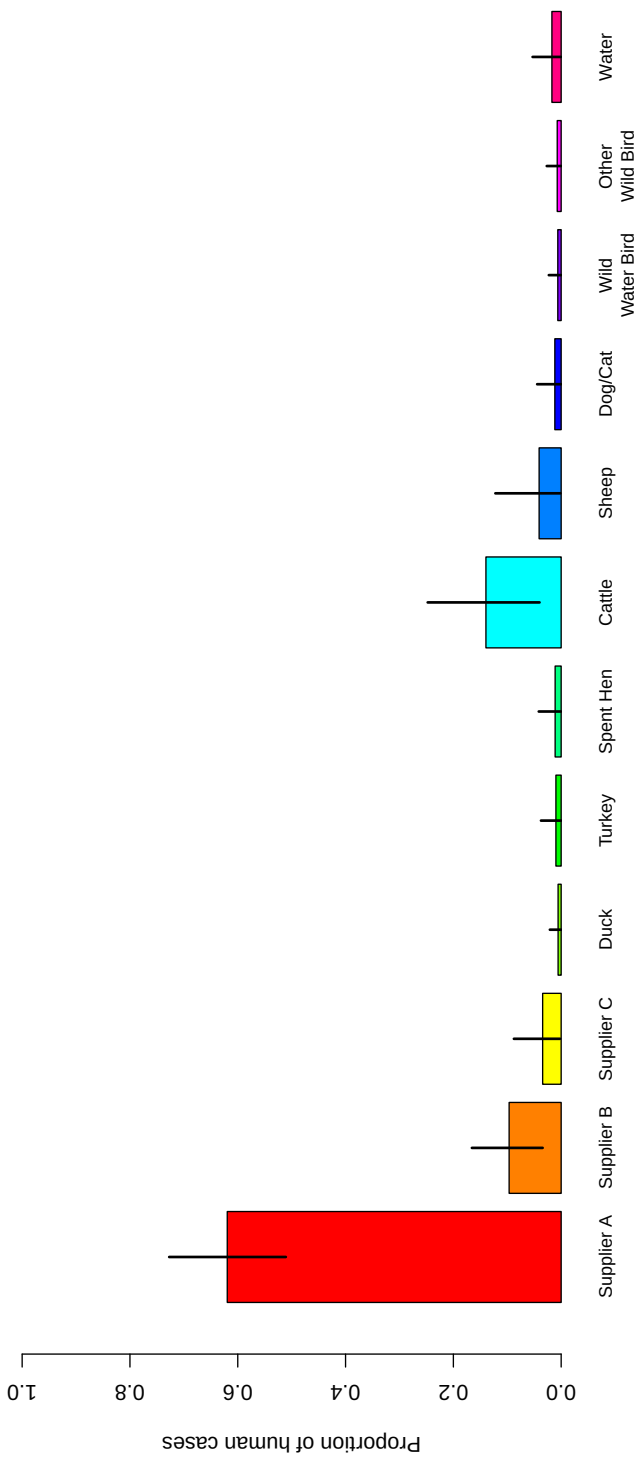

Figure 1: Pre-intervention: Source attribution for human cases in the Manawatu for cases reported between July 1st 2005 and June 30th 2006. Only source data from the Manawatu were used in this analysis. Error bars represent 95% confidence or credible intervals

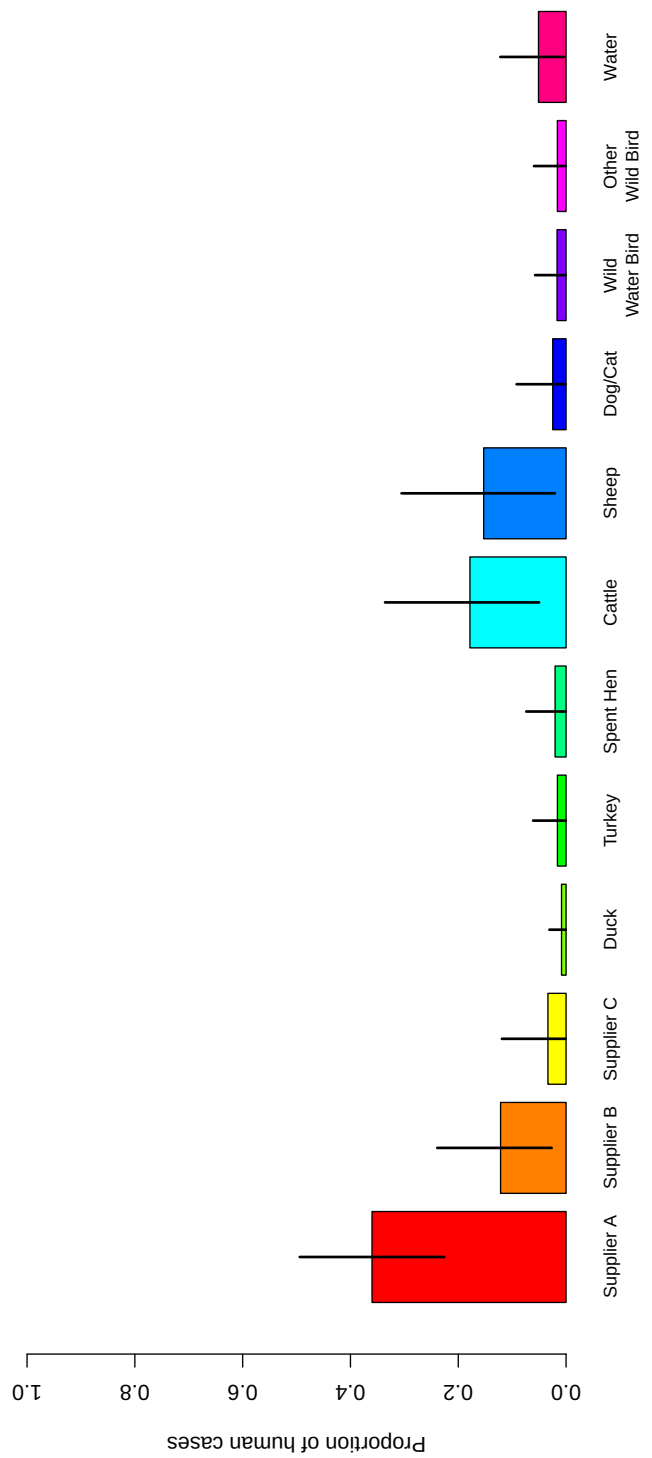

Figure 2: Post-intervention: Source attribution for human cases in the Manawatu for cases reported between July 1st 2009 and June 30th 2010. Only source data from the Manawatu were used in this analysis, including the 2009/10 poultry MLST data. Error bars represent 95% confidence or credible intervals

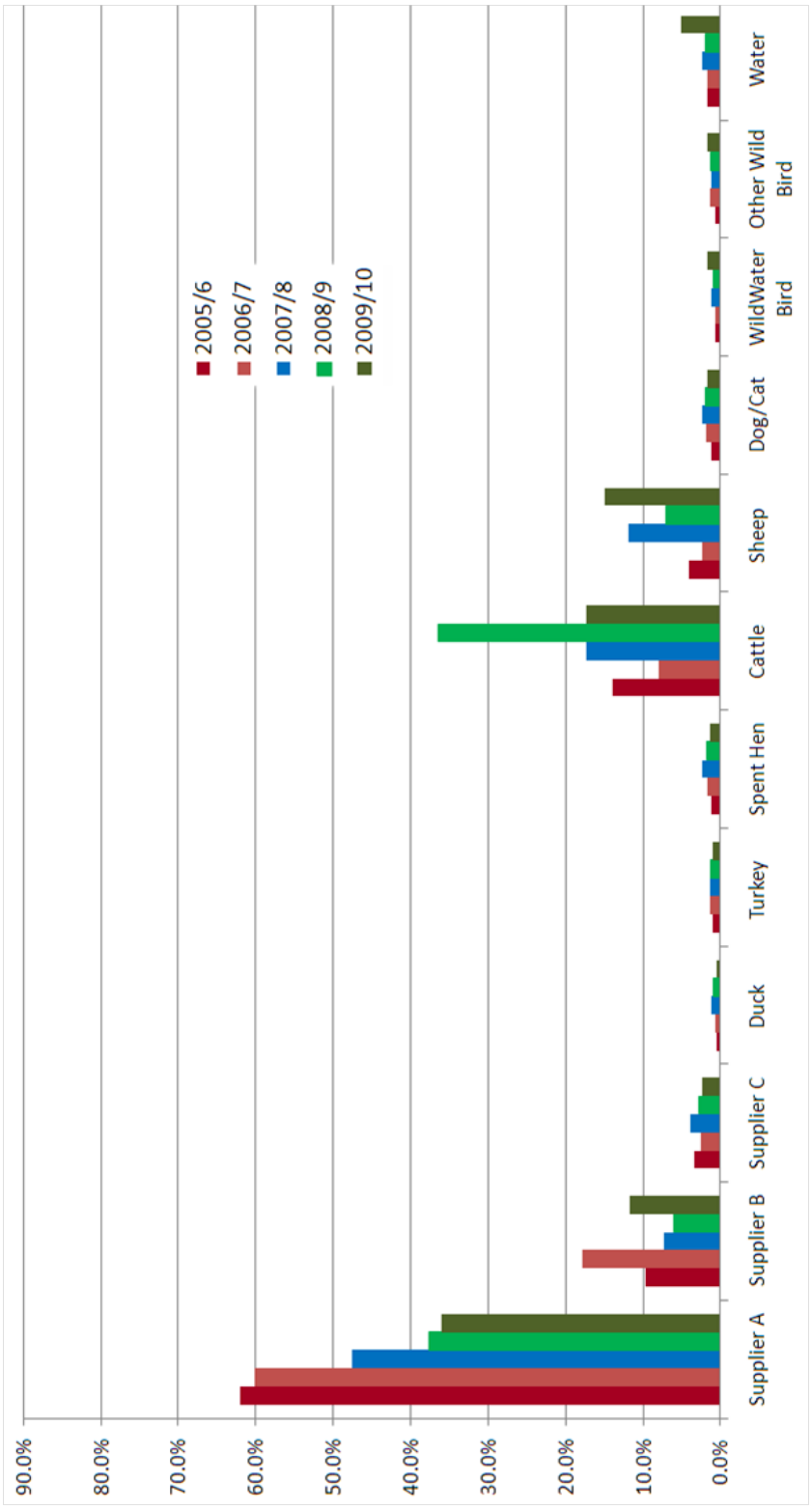

Figure 3: Source attribution estimates for human cases in the Manawatu for 5 twelve monthly periods starting on the 1st July 2005 and ending on the 30th June 2010. The pre-intervention years are shaded red, the transition year in blue and the post-intervention years are shaded green.

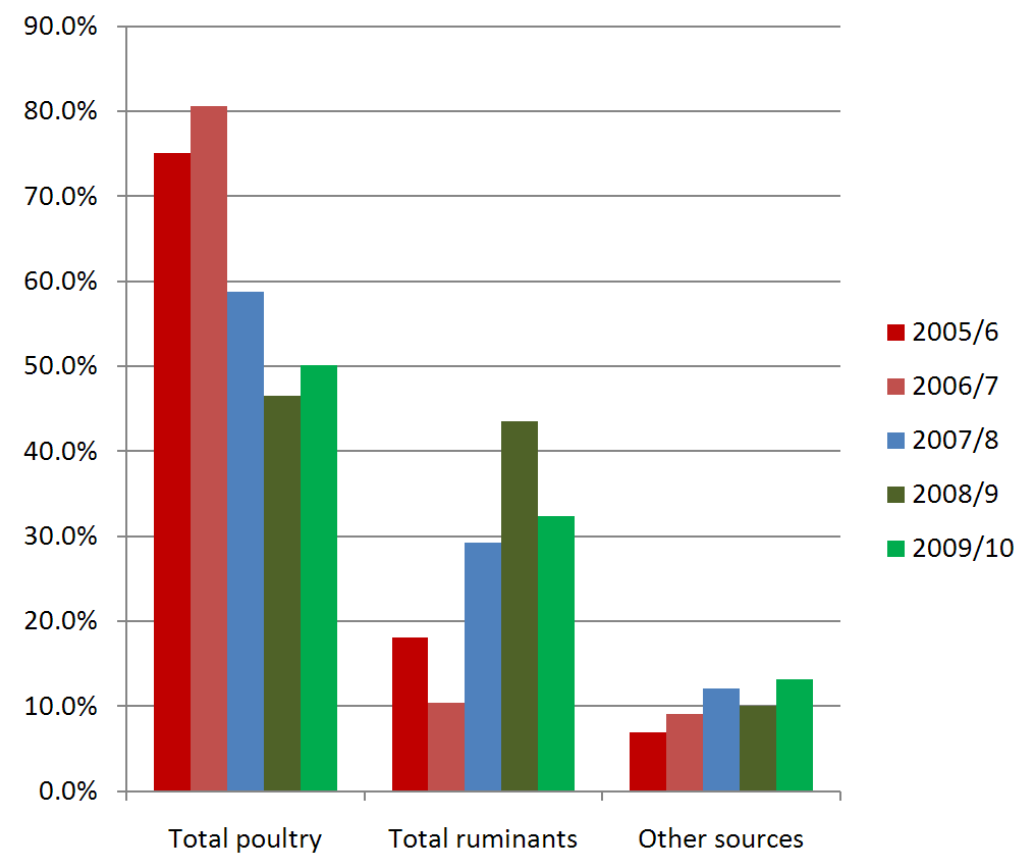

Figure 4: Poultry, ruminant and other source attribution estimates for human cases in the Manawatu for 5 twelve monthly periods starting on the 1st July 2005 and ending on the 30th June 2010. The pre-intervention years are shaded red, the transition year in blue and the post-intervention years are shaded green.

Table 2: The estimated percentage of human cases of campylobacteriosis in the Manawatu attributable to each source for five 12 month periods beginning on the 1st July 2005 and ending on the 30th June 2010. Medians 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.

Source	**2005/6**			**2006/7**			**2007/8**			**2008/9**			**2009/10**		
	Median	2.5%	97.5%	**Median**	2.5%	97.5%	**Median**	2.5%	97.5%	**Median**	2.5%	97.5%	**Median**	2.5%	97.5%
Supplier A	61.9	51.1	72.7	60.2	49.3	70.9	47.6	32.8	62.3	37.7	23.7	51.2	36	22.6	49.4
Supplier B	9.7	3.4	16.6	17.8	9.9	26.4	7.3	0.7	17	6.1	0.7	13.7	11.7	2.7	23.9
Supplier C	3.4	0.2	8.8	2.6	0.1	8.3	3.9	0.1	13.6	2.7	0.1	9.4	2.4	0.1	11.9
Duck	0.6	0	2.1	0.6	0	2.4	1.1	0	4.2	0.8	0	3.1	0.6	0	3.1
Turkey	1	0	3.7	1.3	0	4.7	1.4	0	5.2	1.3	0	4.7	1.1	0	6.1
Spent Hen	1.1	0	4.2	1.8	0	6.4	2.3	0.1	8.5	1.8	0	6.9	1.4	0.1	7.4
Cattle	14	4	24.8	7.9	1.2	16.1	17.4	3.2	33.7	36.4	21.3	52.3	17.3	5	33.6
Sheep	4.1	0.1	12.2	2.4	0.1	8	11.9	1	26.3	7.1	0.3	19.9	15	2.1	30.5
Dog/Cat	1.2	0	4.5	1.8	0	6.5	2.4	0.1	8.7	2	0.1	7.3	1.7	0.1	9.2
WildWater Bird	0.6	0	2.3	0.7	0	2.6	1.2	0	4.3	1	0	3.5	1.7	0	5.8
Other Wild Bird	0.7	0	2.7	1.3	0	4.4	1.2	0	4.3	1.2	0	4.4	1.6	0	5.9
Water	1.7	0.1	5.3	1.6	0	5.5	2.3	0.1	7.7	1.9	0.1	6.6	5.1	0.4	12.2

4.3 Dynamic modelling

Figure 5 shows the temporal attribution results. The summer peaks, most of which are attributed to poultry sources, can be clearly seen, with the peak for summer 2008/2009 approximately half the level of previous years. The dramatic reduction in poultry attribution for the first half of 2008 can be seen to correspond to a reduction in isolates from all sources, although the reduction in poultry is of a higher magnitude. Ruminant strains on the whole appear relatively stable at between 10 and 20 cases per month. Figure 6 shows the number of cases attributed to poultry including 95% credible envelopes, which indicate a moderate amount of uncertainty in the estimates. The peaks of poultry attributed infection in the summer months, and a large dip in poultry attributed cases during 2008, with comparably lower case numbers in the post intervention period are clearly visible.

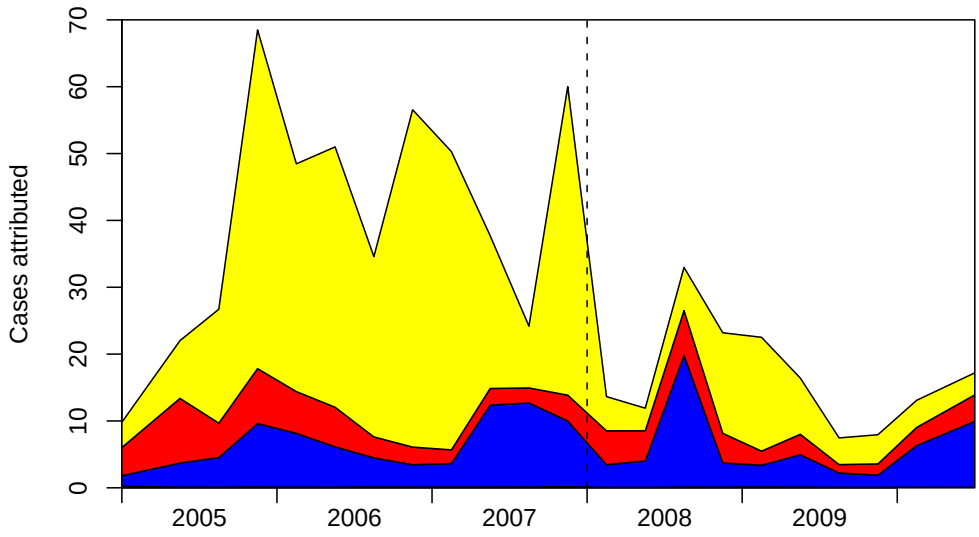

Figure 5: Estimated number of human cases per month attributed to each source by the modified Hald model using three-monthly intervals from 1st March 2005 to June 2010. Colours indicate the source the cases are attributed to: yellow (poultry), cattle (blue) and sheep (red). Other sources contributed to fewer than one case per interval and are therefore not included in the graph. The dashed vertical line represents the cut-off date between the pre- and post intervention periods.

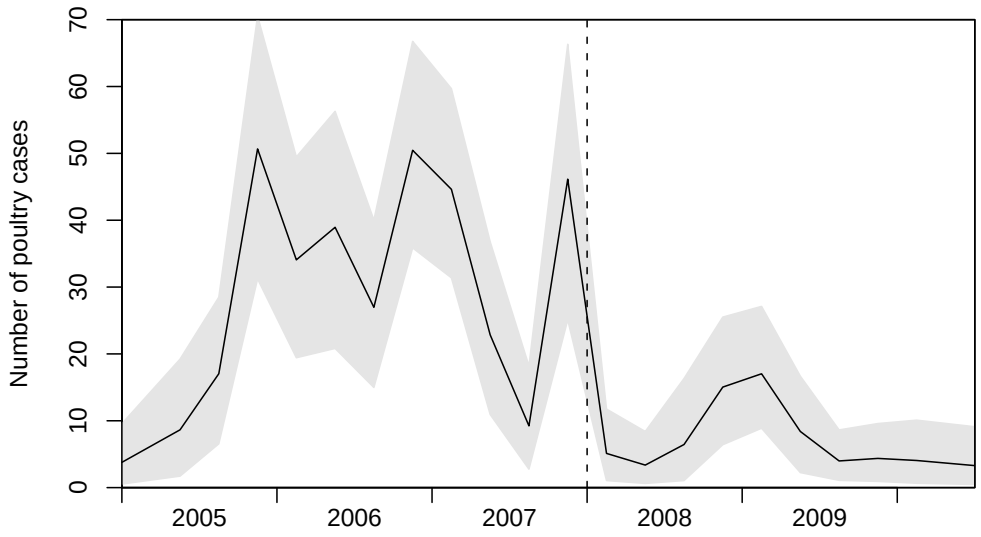

Figure 6: Estimated number of human cases per month attributed to poultry by the modified Hald model with 95% credible intervals using three-monthly intervals from 1st March 2005 to June 2010. The dashed vertical line represents the cut-off date between the pre- and post intervention periods.

5 Discussion and Conclusions

This study provides an update of the source attribution estimates for campylobacteriosis in the Manawatu sentinel surveillance site. The decline in poultry-associated human cases following the interventions in 2006/7 has persisted into 2010. However, poultry, particularly Supplier A, are still estimated to be the most important source of human infection in the region. Although there was an estimated 50% reduction in human case rates across New Zealand (equivalent to a rate ratio of 0.5), the relative reduction varied geographically. The rate ratios comparing 2009 with 2006 and the rankings for each DHB are shown in Table 3. This table shows that a reduction in rates was observed in all DHBs but the largest relative reductions (smallest rate ratios) were generally observed in South Island DHBs. In the MidCentral/Manawatu region the rate ratio was only 0.65 (rate in 2009 divided by the rate in 2006: this is consistent with the decline in poultry associated human cases observed in this study. It is also notable that many of the North Island DHBs were higher in the ranking of campylobacteriosis rates in 2009 compared to 2006. In contrast many South Island DHBs moved to a lower position in the disease rankings.

We conclude that this study has provided evidence that the continued reduction in cases in the Manawatu is attributable to improvements in the poultry supply. However, the relatively low reduction in case rates in MidCentral/Manawatu suggest that these improvements have not been as successful as those observed in other regions of New Zealand.

Table 3: Rates per 100,000 population of human cases of campylobacteriosis in DHBs in New Zealand, including the MidCentral / Manawatu area. Rate ratios compare the rate in 2009 with the rate in the pre-intervention year: 2006. Data were taken from ESR Annual Surveillance reports.

	2006			2009			2009 compared to 2006	
DHB	Number of cases	Rate	Rank	Number of cases	Rate	Rank	Change in rank	Rate ratio
Wairarapa	89	226.9	19	69	172.6	11	+8	0.76
Mid Central	359	219.9	20	236	142.3	14	+6	**0.65**
Hawke's Bay	518	344	14	336	218.3	4	+10	0.63
Tairawhiti	58	130.3	21	36	77.9	21	0	0.60
Whanganui	233	375.2	11	139	220.1	3	+8	0.59
Hutt	606	437.9	5	355	248.8	1	+4	0.57
Northland	374	249.9	17	208	133.5	17	0	0.53
West Coast	74	242.5	18	42	128.9	18	0	0.53
Waikato	1220	356.3	12	661	183.6	8	+4	0.52
Lakes	410	403.5	8	205	201.4	6	+2	0.50
Nelson Marlborough	359	262.6	16	173	126.5	19	-3	0.48
Taranaki	472	448.8	4	233	215.3	5	-1	0.48
Capital and Coast	1425	510.6	2	692	240.2	2	0	0.47
Bay of Plenty	650	327.1	15	317	152.6	12	+3	0.47
Auckland	1813	421.1	6	810	182.4	9	-3	0.43
Counties Manukau	1548	350.3	13	685	142.2	15	-2	0.41
Southland	415	379.6	10	165	147.6	13	-3	0.39
Waitemata	2319	462.4	3	913	172.8	10	-7	0.37
South Canterbury	285	531.7	1	103	185.4	7	-6	0.35
Otago	742	406.2	7	253	134.2	16	-9	0.33
Canterbury	1904	398.4	9	545	108.6	20	-11	0.27

6 Acknowledgment

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A Source attribution estimates from the island model for intervening years

Graphical outputs from the island model including 95% credible intervals for each year between July 1st 2006 and June 30th 2009 are shown in Figures 7, 8 and 9.

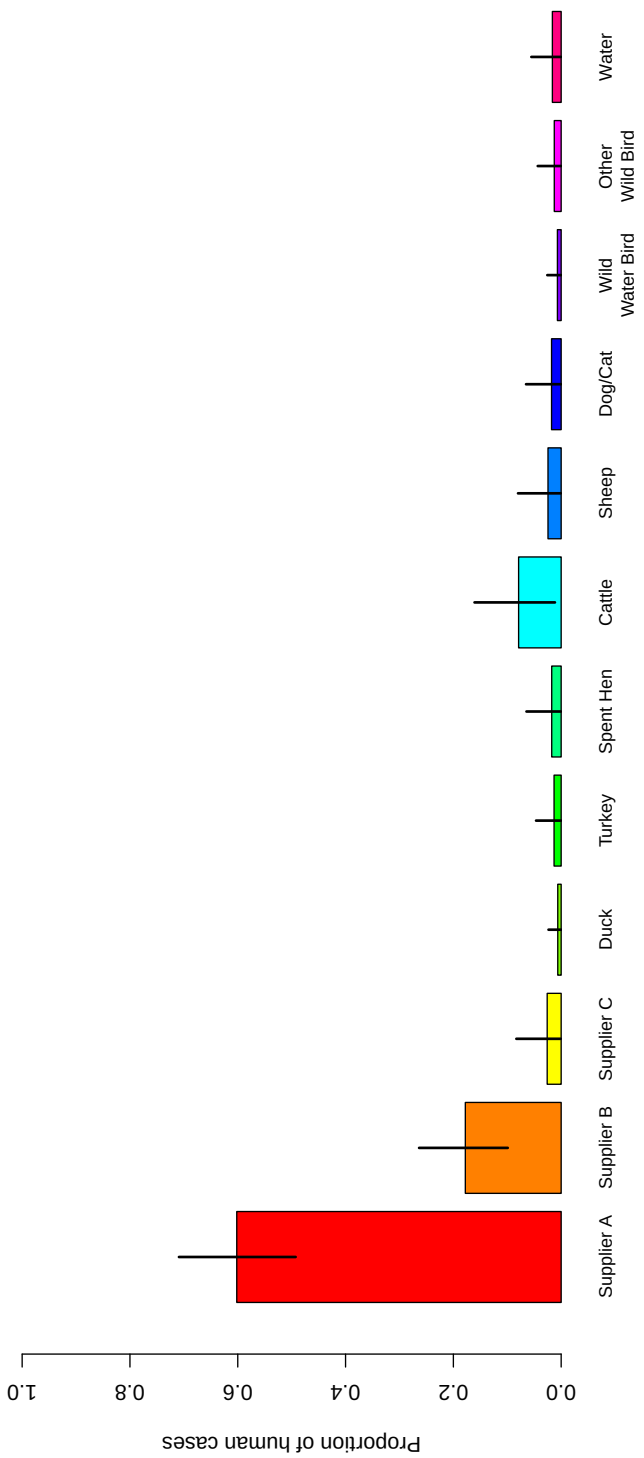

Figure 7: Pre-intervention: Source attribution for human cases in the Manawatu for cases reported between July 1st 2006 and June 30th 2007. Only source data from the Manawatu were used in this analysis. Error bars represent 95% confidence or credible intervals

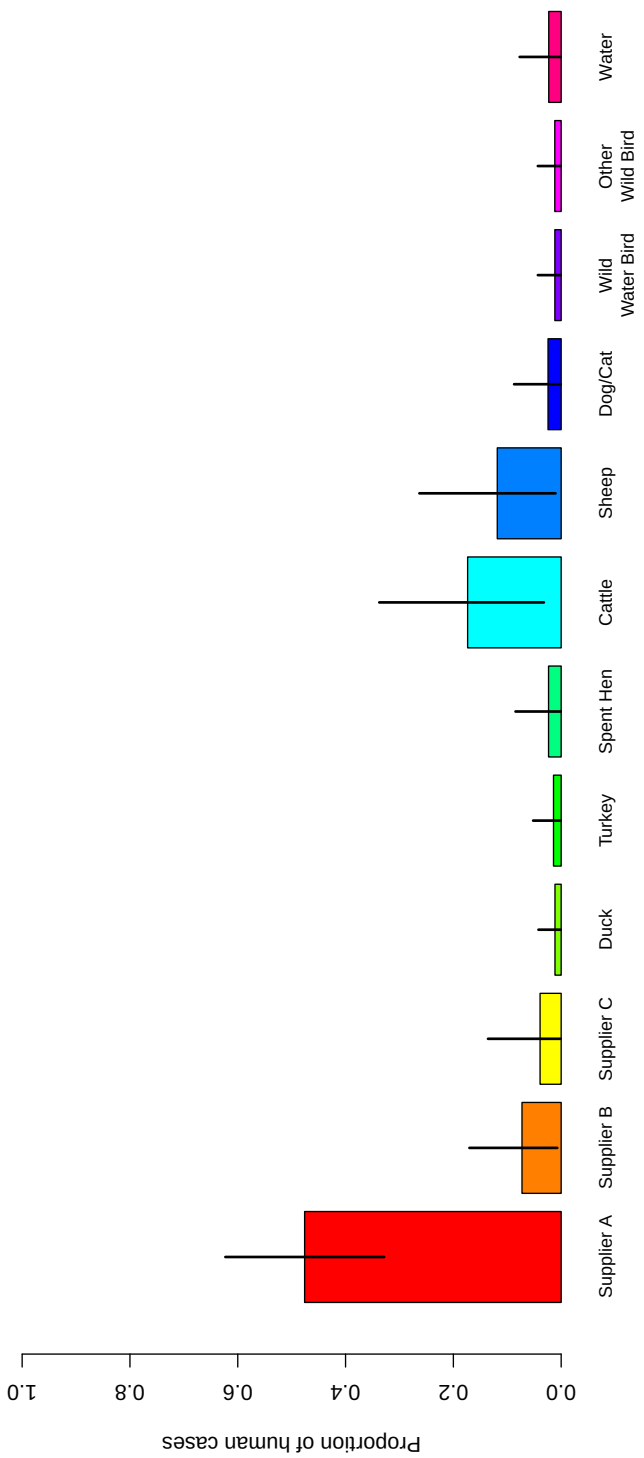

Figure 8: Intervention transition year: Source attribution for human cases in the Manawatu for cases reported between July 1st 2007 and June 30th 2008. Only source data from the Manawatu were used in this analysis. Error bars represent 95% confidence or credible intervals

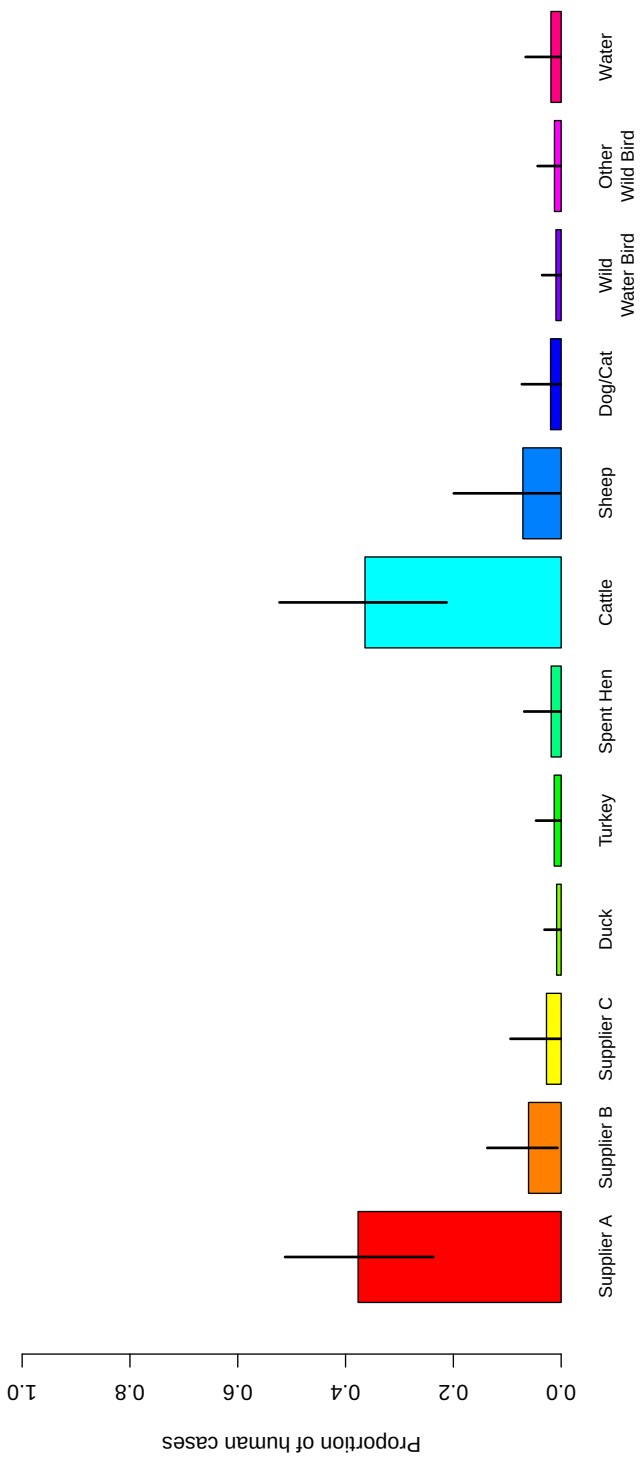

Figure 9: Post-intervention: Source attribution for human cases in the Manawatu for cases reported between July 1st 2008 and June 30th 2009. Only source data from the Manawatu were used in this analysis. Error bars represent 95% confidence or credible intervals

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