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Assigning the source of human campylobacteriosis in New Zealand: A comparative genetic and epidemiological approach

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ABSTRACT

Integrated surveillance of infectious multi-source diseases using a combination of epidemiology, ecology, genetics and evolution can provide a valuable risk-based approach for the control of important human pathogens. This includes a better understanding of transmission routes and the impact of human activities on the emergence of zoonoses. Until recently New Zealand had extraordinarily high and increasing rates of notified human campylobacteriosis, and our limited understanding of the source of these infections was hindering efforts to control this disease. Genetic and epidemiological modeling of a 3-year dataset comprising multilocus sequence typed isolates from human clinical cases, coupled with concurrent data on food and environmental sources, enabled us to estimate the relative importance of different sources of human disease. Our studies provided evidence that poultry was the leading cause of human campylobacteriosis in New Zealand, causing an estimated 58–76% of cases with widely varying contributions by individual poultry suppliers. These findings influenced national policy and, after the implementation of poultry industry-specific interventions, a dramatic decline in human notified cases was observed in 2008. The comparative-modeling and molecular sentinel surveillance approach proposed in this study provides new opportunities for the management of zoonotic diseases.

1. Introduction

Emerging zoonotic pathogens, including *Campylobacter* spp., are commonly associated with a wide taxonomic and ecological host range, and are more likely to infect both domestic and wild animals (Woolhouse, 2002). Studies of the population structure and biology of pathogens with multiple hosts can improve our knowledge of their complex transmission ecology, advance models of infectious diseases, and thereby inform control strategies. In general, disease transmission, and thereby the exposure of susceptible hosts to potential pathogens, is affected by changes in the host pathogen ecology. New opportunities for pathogen transmission may arise following changes in, for example, land use, climate, host demography and food production practices (Woolhouse, 2002). Pathogen characteristics such as virulence and host association are the result of complex interactions involving evolutionary, ecological and epidemiological processes. Merging epidemiology with evolutionary ecology can therefore improve our understanding of the evolution and emergence of pathogens and help guide public health policy (Galvani, 2003).

Knowledge of the proportion of human cases of zoonotic disease that are caused by a particular exposure source is critical for the prioritization of public health resources and the successful implementation of control measures (Batz et al., 2005). Bacterial source tracking has been identified as a tool to link people who are ill to the sources of bacterial contamination (Foley et al., 2009), and new molecular tools are being increasingly applied to study transmission patterns within populations at the strain level, and to evaluate host- and strain-specific risk factors (Murray, 2002). However, when there are many risk pathways and multiple hosts and sources of infection, estimating the relative contribution of different pathogen reservoirs to human infection is challenging.

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Table 1
Overview of samples collected in the Manawatu, New Zealand, 2005–2008.

Source	Samples collected	Samples culture positive[a]	Samples confirmed *C. jejuni*[b]	Samples typed and included in analysis[c]
Human cases	773	661	584	502
Fresh chicken carcasses	562	454	415	275
Retail meats beef	400	44	33	29
Retail meats lamb	418	112	103	87
On-farm cattle	145	119	80	66
On-farm sheep	133	92	61	49
Environmental water	335	140	82	70
Total samples	2766	1622	1215	969

[a] A sample was considered culture positive for *Campylobacter* spp., when colonies showed typical *Campylobacter* morphology on mCCDA and BA.
[b] A sample was considered *C. jejuni* positive, when at least one isolate from this sample was confirmed as *C. jejuni* by PCR.
[c] This refers to the number of samples with a complete MLST profile.

Recently several advances have been made in our understanding of the evolution and population structure of *Campylobacter* spp., most notably following the development and application of a multilocus sequence typing (MLST) scheme for this pathogen. These advances include the identification of potentially environmentally adapted *Campylobacter jejuni* strains (Sopwith et al., 2008), and comparisons of the population biology and molecular biology of *C. jejuni* and *C. coli*, which have provided evidence for the ecologically driven convergence of these two pathogens (Dingle et al., 2005; Sheppard et al., 2008).

The availability of molecular typing schemes, combined with new modeling tools based on different underlying assumptions, have provided a platform for understanding the origin of human infections and informing public health policy. For example the model described by Hald et al. (2004) is an associative simulation model for salmonellosis that utilizes the distribution of bacterial subtypes in potential sources of disease to estimate the contribution of each source to the human disease burden. The model accounts for differences in the ability of sources to transmit disease and differences in virulence, pathogenicity and survival of pathogen subtypes. Subsequently this tool has been modified and applied to other pathogens including *C. jejuni* (Mullner et al., 2009). In addition a new generation of genetic attribution tools have been developed and applied to campylobacteriosis (Wilson et al., 2008; Sheppard et al., 2009; Strachan et al., 2009). These models use the relative frequency and relatedness of isolates from different sources to infer attribution estimates. By taking a population-genetics approach the evolutionary relationships between pathogen populations can be better understood and key reservoirs can be identified.

New Zealand is used as a study case to illustrate our approach. Campylobacteriosis has emerged as a major public health problem worldwide and in New Zealand the number of notifications has increased markedly over the last decade. In 2005 and 2006 the incidence exceeded 300 cases per 100,000 people per annum (Baker et al., 2006). In consequence the relatively high prevalence of campylobacteriosis in New Zealand attracted considerable media attention, was regarded as a national epidemic, and raised a public demand for urgent action (Baker et al., 2006). However, the complex epidemiology of campylobacteriosis, and lack of an appropriate subtyping scheme has hindered the development of successful measures to control this emerging pathogen in New Zealand and elsewhere (Mullner et al., 2009). The situation in New Zealand is quite unique (Crump et al., 2001): the country is geographically remote, with extensive and changing agricultural land use, and human, animal and pathogen populations that are relatively isolated. Although there is relatively little importation of animals and animal products into the country as a result of rigid border biosecurity measures, the country is exposed to a large number of international travelers. Of particular relevance to *Campylobacter* spp., and in contrast to many other countries; the country's poultry suppliers focus almost entirely on the domestic market and, for biosecurity reasons, no raw poultry products are imported into the country. These factors are likely to play an important role in the invasion, dissemination and evolution of multi-host pathogens such as *C. jejuni* and *C. coli*.

The use of integrated surveillance, across human, domestic animal and wildlife populations has been identified as a key component of strategies aimed at preventing and controlling emerging pathogens, in particular since the population dynamics of multi-host pathogens is often poorly understood (Woolhouse, 2002). In this study samples from human clinical cases, animal-derived food products and the environment were gathered in a defined geographical area of New Zealand over a 3-year period (French and Molecular Epidemiology and Veterinary Public Health Group Hopkirk Institute, 2008) and genotyped using MLST (Dingle et al., 2002). The resulting dataset contained a total of 969 typed samples of which 502 were from human cases. The temporal and spatial scale of this study allowed for a more complete understanding of local transmission dynamics compared with previous research (Wilson et al., 2008; Sheppard et al., 2009). The objective of this study was to estimate the relative contribution of food and non-food sources to the burden of human campylobacteriosis in New Zealand and test the hypothesis that poultry rather than ruminants, wildlife and water were the predominant reservoir for human infection (Savill et al., 2001; Devane et al., 2005; Nelson and Harris, 2006).

2. Materials and methods

2.1. Sampling

Over a 3-year period from March 1st 2005 until February 29th 2008 a total of 2766 human, retail meat, on-farm and environmental samples were collected in the Manawatu region of New Zealand's North Island (French and Molecular Epidemiology and Veterinary Public Health Group Hopkirk Institute, 2008). This included monthly sampling of retail fresh carcasses from different poultry suppliers in the region, which are dominated by two major suppliers named A[2] and B in this study. Two smaller companies share the remaining part of the market ("Supplier C"). The dataset used for the attribution models consists of a subset of confirmed *C. jejuni* samples from the above mentioned study and wild bird samples from a separate study (French et al., 2008). The aim was to get a subset of approximately 100 isolates from each source spread over the whole study period and the resulting dataset contained a total of 969 typed samples of which 502 were from human cases (Table 1). Human surveillance information was linked to laboratory notified cases and duplicates. Travelers and out of region cases were excluded from the dataset.

[2] Supplier identification letters were assigned arbitrarily.

2.2. Bacterial culture and identification

Human 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.

Chickens were washed and massaged in 200 ml of Buffered Peptone Water (BPW) (Difco, USA). The chicken wash was centrifuged (16,264 RCF (g), 6 °C, 35 min, 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 Broth, which was incubated at 42 °C microaerobically for 2 days. After incubation, the broth was subcultured onto mCCDA and incubated microaerobically at 42 °C for 2 days.

For red meat sampling, 10 g (±1 g) of mince or liver was aseptically removed from the packet and stomached briefly with 90 ml of Bolton Broth. The Bolton Broth plus meat was incubated at 42 °C microaerobically for 2 days. After incubation the broth was subcultured onto mCCDA and incubated microaerobically at 42 °C for another 2 days.

For the environmental water samples, the broth containing the filter was incubated at 42 °C microaerobically for 2 days. After incubation, the broth was subcultured onto mCCDA and incubated microaerobically at 42 °C for 2 days.

The swabs from the cattle and sheep environmental sites were immersed together in 20 ml of Bolton Broth and incubated microaerobically at 42 °C for 2 days. After incubation the broth was subcultured onto mCCDA and incubated microaerobically at 42 °C for 2 days.

Water samples were collected in sterile 200 ml bottles from defined sites around the Manawatu. Samples were transported in the dark to the laboratory and 100 ml aliquots were filtered through sterile 0.4 μm filters (Whatman, USA). Filters were immersed in 20 ml of Bolton Broth and incubated microaerobically at 42 °C for 2 days. After incubation the broth was subcultured onto mCCDA and incubated microaerobically at 42 °C for 2 days.

From all sources single colonies resembling *Campylobacter* species were subcultured to BA and incubated microaerobically at 42 °C for 2 days before DNA preparations were made. Cultures were frozen at −80 °C.

Isolates of *Campylobacter* genus were identified by the method outlined by Linton et al. (1996). Isolates were further speciated by using the *map*A gene that was shown to be found only in *C. jejuni* (Stucki et al., 1995). Primers MapA-F (5′-CTTGGCTTGAAATTTGCTTG-3′) and MapA-R (5′-GCTTGGTGCGGATTGTAAA-3′) were designed to target this gene for speciation. Amplification protocols for pan-*Campylobacter* and the *map*A genes were based on the methods outlined by Linton et al. (1996) and Stucki et al. (1995) respectively, with slight modifications. The PCR products were visualised by electrophoresis in a 1% agarose gel in TBE buffer, which was then stained with ethidium bromide and exposed to UV light. The presence of a 603 bp product indicated *C. jejuni*.

2.3. Genotyping

After speciation, MLST of *C. jejuni* isolates was performed using seven house-keeping genes: *aspA* (aspartase A), *glnA* (glutamine synthase), *gltA* (citrate synthase), *glyA* (serine hydroxymethyltransferase), *pgm* (phosphoglucomutase), *tkt* (transketolase) and *uncA* (ATP synthase alpha subunit) based on the method as outlined by Dingle et al. (2001). Chromosomal DNA was prepared from freshly grown cultures by boiling a small loopful in 2% Chelex™ (Bio-Rad Laboratories, CA, USA) for 10 min followed by centrifugation of the disrupted cells. The supernatant was decanted to a fresh tube and used for amplification. The amplifications were performed in a 25 μl volume reaction using Applied Biosystems AmpliTaq Gold mastermix (Applied Biosystems, Auckland New Zealand) and 5 pmoles of each primer. Products were sequenced on an ABI 3130XL automated DNA sequencer using ABI BigDye v3.1 (Applied Biosystems) following the manufacturer's instructions. Sequence data were collated and alleles assigned using the *Campylobacter* PubMLST database (http://pubmlst.org/campylobacter/). Novel alleles and sequence types (ST) were submitted for allele and ST designation as appropriate. Alleles that did not give clear results were re-amplified and sequenced using primers sets published by Miller et al. (2005) using the same protocol as above. Table 2 shows the dataset of MLST typed samples, which was used in this study to allocate human cases to a source.

2.4. Analysis

2.4.1. Proportional similarity index

The proportional similarity index (PSI) or Czekanowski index is an objective and simple measure of the area of intersection between two frequency distributions (Rosef et al., 1985) that can estimate the similarity between the frequency distributions of bacterial subtypes from different sources. The PSI is calculated by: $\mathrm{PSI} = 1 - 0.5\sum_i |p_i - q_i| = \sum_i \min(p_i, q_i)$, where p_i and q_i represent the proportion of strains belonging to type i out of all strains typed from sources P and Q (Feinsinger et al., 1981; Rosef et al., 1985). The value for PSI ranges between one for identical frequency distributions, to zero for distributions with no common types. Bootstrap confidence intervals for this measure were estimated (Garrett et al., 2007) and the analysis was implemented in R, version 2.7.0 (R Development Core Team, 2005).

2.4.2. Dutch model

The principle behind the Dutch model (Van Pelt et al., 1999) is to compare the number of reported human cases caused by a particular bacterial subtype with the relative occurrence of that subtype in each source. The number of reported cases per subtype and source is estimated by:

$$\lambda_{ij} = \frac{p_{ij}}{\sum_j p_{ij}} x_i,$$

where p_{ij} is the relative occurrence of bacterial subtype i in source j, x_i is the estimated number of human cases of type i per year and λ_{ij} is the expected number of cases per year of type i from source j. A summation across subtypes gives the total number of cases from source j, denoted by λ_j: $\lambda_j = \sum_i \lambda_{ij}$. The method of Garrett et al. (2007) was extended to provide bootstrap confidence intervals for the Dutch model and the model was implemented in R, version 2.7.0 (R Development Core Team, 2005).

2.4.3. Modified Hald model

Similar to the Dutch model, the modified Hald model compares the number of human cases caused by different bacterial subtypes with their prevalence in different food sources. However, by using a Bayesian approach, the Hald model can explicitly include and quantify the uncertainty surrounding each of the parameters (Mullner et al., 2009). In the model o_i represents the number of human cases of type i and the expected number of cases of *Campylobacter* type i from source j is denoted by λ_{ij}. Assume that

$$o_i \sim \mathrm{Poisson}(\Sigma_j \lambda_{ij}), \quad (1)$$

and that

$$\lambda_{ij} = p_{ij} q_i a_j, \quad (2)$$

Table 2
Relative frequency of sequence types (ST) in % and total number of isolates from human cases and disease sources in the Manawatu, New Zealand, 2005–2008.

ST	Source type						
	Human	Supplier A	Supplier B	Supplier C	Bovine	Ovine	Environment
21	1.4	0.0	0.8	0.0	4.0	0.7	0.0
25	0.2	1.5	0.0	2.5	0.0	0.0	1.1
38	2.6	0.0	0.0	0.0	1.0	0.0	0.0
42	3.8	3.1	0.0	0.0	11.1	12.9	1.1
45	8.2	27.5	12.6	28.4	2.0	1.4	17.8
48	8.4	0.8	22.7	8.6	0.0	0.0	0.0
50	4.6	3.8	17.6	16.0	12.1	15.7	1.1
52	3.4	4.6	0.0	0.0	0.0	0.0	0.0
53	5.4	7.6	3.4	3.7	24.2	0.7	0.0
61	2.8	0.0	0.0	0.0	8.1	10.0	1.1
81	0.2	0.0	0.0	0.0	0.0	0.0	0.0
137	0.2	0.0	0.0	0.0	0.0	0.0	2.2
177	0.0	0.0	0.0	0.0	0.0	0.0	2.2
190	4.2	6.1	0.0	0.0	7.1	5.7	0.0
219	0.2	0.0	0.0	0.0	0.0	0.0	0.0
227	0.0	0.8	0.0	0.0	0.0	0.0	0.0
257	2.4	7.6	3.4	0.0	0.0	0.0	0.0
354	4.6	2.3	0.0	1.2	0.0	0.0	0.0
393	0.0	0.0	0.0	0.0	0.0	0.7	0.0
403	0.2	0.0	0.0	0.0	0.0	0.0	0.0
422	0.6	0.0	0.0	0.0	2.0	13.6	3.3
436	0.8	0.0	0.0	0.0	2.0	2.1	2.2
451	1.6	0.0	0.0	24.7	0.0	0.0	0.0
459	0.2	0.0	0.0	0.0	0.0	0.0	0.0
474	30.7	20.6	1.7	0.0	5.1	1.4	2.2
520	1.6	3.8	0.0	2.5	6.1	2.1	0.0
526	0.0	0.0	0.0	0.0	0.0	0.0	1.1
578	0.2	0.0	0.0	0.0	0.0	0.0	0.0
583	1.8	6.1	0.0	4.9	0.0	0.7	1.1
618	0.0	0.0	0.0	0.0	0.0	0.7	0.0
658	0.4	0.0	0.0	0.0	0.0	0.0	0.0
677	1.0	0.0	0.8	0.0	0.0	0.7	1.1
694	0.0	0.0	0.0	0.0	0.0	0.0	1.1
829	0.2	0.0	0.0	0.0	0.0	0.0	0.0
1030	0.0	0.0	0.0	0.0	0.0	0.0	1.1
1115	0.0	0.0	0.0	0.0	1.0	0.0	0.0
1191	0.0	0.0	0.0	0.0	0.0	0.7	0.0
1223	0.0	0.0	0.0	0.0	0.0	0.0	1.1
1225	0.0	0.0	0.0	0.0	0.0	0.0	7.8
1243	0.0	0.0	0.0	0.0	0.0	0.0	1.1
1457	0.2	0.0	0.0	0.0	0.0	0.0	0.0
1517	0.8	0.0	5.0	0.0	0.0	5.0	0.0
1581	0.2	0.0	4.2	0.0	0.0	0.0	0.0
1707	0.2	0.0	0.0	0.0	0.0	0.0	0.0
1818	0.0	0.0	0.8	0.0	0.0	0.0	0.0
1911	0.0	0.0	0.8	0.0	0.0	0.0	0.0
2026	2.2	0.0	0.0	0.0	5.1	12.9	0.0
2219	0.2	0.0	0.0	0.0	0.0	0.0	0.0
2343	0.2	0.0	0.0	0.0	0.0	0.0	0.0
2345	0.6	0.8	9.2	0.0	0.0	0.0	0.0
2347	0.0	0.0	0.0	0.0	0.0	0.0	1.1
2350	0.4	0.0	0.0	0.0	1.0	0.0	0.0
2354	0.0	0.0	0.0	0.0	0.0	0.0	1.1
2381	0.0	0.0	0.0	0.0	0.0	0.0	17.8
2391	0.2	0.8	0.0	0.0	0.0	0.0	0.0
2392	0.0	0.0	0.0	0.0	0.0	0.7	0.0
2397	0.0	0.0	2.5	0.0	0.0	0.0	0.0
2535	0.0	0.0	0.0	1.2	0.0	0.0	0.0
2584	0.0	0.0	0.0	0.0	0.0	0.0	1.1
2619	0.0	0.0	0.0	0.0	0.0	0.0	1.1
3072	0.2	0.0	0.0	0.0	2.0	0.0	0.0
3222	0.2	0.0	0.0	0.0	0.0	0.0	0.0
3230	0.0	0.0	0.0	1.2	0.0	0.0	0.0
3232	0.0	0.0	0.0	0.0	0.0	1.4	0.0
3301	0.0	0.0	0.0	0.0	0.0	0.0	1.1
3538	0.2	0.0	0.0	0.0	0.0	0.0	1.1
3609	0.0	0.0	12.6	0.0	0.0	0.0	0.0
3610	0.0	0.0	0.0	0.0	0.0	1.4	1.1
3640	0.0	0.0	0.0	0.0	0.0	0.0	1.1
3655	0.0	0.0	0.0	0.0	0.0	0.0	2.2
3656	0.0	0.0	0.0	0.0	0.0	0.0	1.1
3657	0.0	0.0	0.0	0.0	0.0	0.0	1.1
3658	0.0	0.0	0.0	0.0	0.0	0.0	1.1
3659	0.0	0.0	0.0	0.0	0.0	0.0	2.2

Table 2 (*Continued*)

ST	Source type						
	Human	Supplier A	Supplier B	Supplier C	Bovine	Ovine	Environment
3660	0.0	0.0	0.0	0.0	0.0	0.0	1.1
3661	0.0	0.0	0.0	0.0	0.0	0.0	1.1
3662	0.0	0.0	0.0	0.0	0.0	0.0	2.2
3663	0.0	0.0	0.0	0.0	0.0	0.0	2.2
3664	0.0	0.0	0.0	0.0	0.0	0.0	1.1
3672	0.0	0.0	0.0	0.0	0.0	0.0	1.1
3673	0.0	0.0	0.0	0.0	0.0	0.0	1.1
3674	0.0	0.0	0.0	0.0	0.0	0.0	1.1
3675	0.0	0.0	0.0	0.0	0.0	0.0	1.1
3676	0.4	0.0	0.0	0.0	0.0	0.7	1.1
3711	0.4	0.0	0.0	1.2	0.0	1.4	0.0
3712	0.4	0.0	0.0	0.0	0.0	0.0	0.0
3714	0.0	0.0	0.0	0.0	1.0	0.0	0.0
3715	0.2	0.0	0.0	0.0	0.0	0.0	0.0
3716	0.0	0.0	0.0	0.0	1.0	0.0	0.0
3717	0.2	0.0	0.0	3.7	0.0	0.0	0.0
3718	0.2	0.0	0.0	0.0	0.0	0.0	0.0
3719	0.0	0.0	0.8	0.0	0.0	0.7	0.0
3720	0.2	0.0	0.0	0.0	0.0	0.0	0.0
3721	0.0	0.8	0.0	0.0	0.0	0.0	0.0
3722	0.0	0.0	0.0	0.0	0.0	0.7	0.0
3723	0.0	0.0	0.0	0.0	1.0	0.0	0.0
3724	0.0	0.0	0.0	0.0	0.0	0.7	0.0
3725	0.0	0.0	0.8	0.0	0.0	0.0	0.0
3726	0.0	1.5	0.0	0.0	0.0	0.0	0.0
3727	0.2	0.0	0.0	0.0	0.0	0.0	0.0
3784	0.2	0.0	0.0	0.0	0.0	0.0	0.0
3792	0.2	0.0	0.0	0.0	0.0	0.0	0.0
3793	0.0	0.0	0.0	0.0	0.0	1.4	0.0
3794	0.0	0.0	0.0	0.0	0.0	0.7	0.0
3795	0.0	0.0	0.0	0.0	0.0	0.7	0.0
3797	0.0	0.0	0.0	0.0	0.0	1.4	0.0
3798	0.0	0.0	0.0	0.0	1.0	0.0	0.0
3799	0.0	0.0	0.0	0.0	2.0	0.0	0.0
3800	0.0	0.0	0.0	0.0	0.0	0.0	1.1
3802	0.0	0.0	0.0	0.0	0.0	0.0	1.1
Isolates	502	131	119	81	99	140	90

where λ_{ij} is the expected number of cases/year of type i from source j, p_{ij} is the prevalence of type i in source j, q_i is the bacteria dependent factor for type i and a_j is the food source dependent factor for type j. The modified Hald model was implemented in WinBUGS 14 (Lunn et al., 2000) and called from R, version 2.7.0 using the R2WinBUGS package (R Development Core Team, 2005).

2.4.4. Asymmetric island model

This Bayesian approach is fundamentally different from the Dutch and Hald models in that it explicitly models the DNA sequence evolution and zoonotic transmission between host species and the environment and thereby assigns human cases probabilistically to source populations (Wilson et al., 2008). This coalescent based model uses an evolutionary approach based on Wright's island model (Wright, 1931). The source of human infection is inferred by assuming that each human isolate represents a sample of one of these source populations and combining information of population structure across all isolates. By modeling the evolutionary processes of mutation and recombination, the approach allows for the occurrence of novel alleles, and novel combinations of alleles, in the human isolates not observed in samples from the source populations.

3. Results

3.1. Proportional similarity index

The genotype distribution of Poultry Supplier A was significantly more similar to the distribution of genotypes from human cases than to that from any other source (median estimate 0.58, 95% CI 0.48–0.64) (Table 3). The PSI was similar for the other two poultry suppliers and bovine sources, with median values between 0.32 and 0.34. The least similarity was observed between environmental and human sources (median estimate 0.18, 95% CI 0.12–0.22).

3.2. Dutch model

The Dutch model (Fig. 2) estimated that the majority of human cases came from the three major poultry suppliers, with Supplier A associated with the most human cases: an estimated 34% (95% CI 28–41%). Bovine sources were estimated to cause 19% of cases (95% CI 14–23%) followed by environmental and ovine sources which were estimated to contribute 12% and 11% of cases respectively.

3.3. Modified Hald model

Poultry Supplier A was estimated by a modified Hald model (Fig. 2) to cause the majority of human cases (58%, 95% CrI 25–78%).

Table 3
The proportional similarity index for each source compared to the distribution of human genotypes, with 95% bootstrapped confidence intervals. Higher values indicate a strong similarity between the genotypes identified in the source and the human cases.

Source	PSI	Lower 95% CI	Upper 95% CI
Poultry Supplier A	0.58	0.48	0.64
Poultry Supplier B	0.32	0.26	0.36
Poultry Supplier C	0.32	0.24	0.35
Bovine sources	0.34	0.28	0.39
Ovine sources	0.28	0.22	0.32
Environmental sources	0.18	0.12	0.22

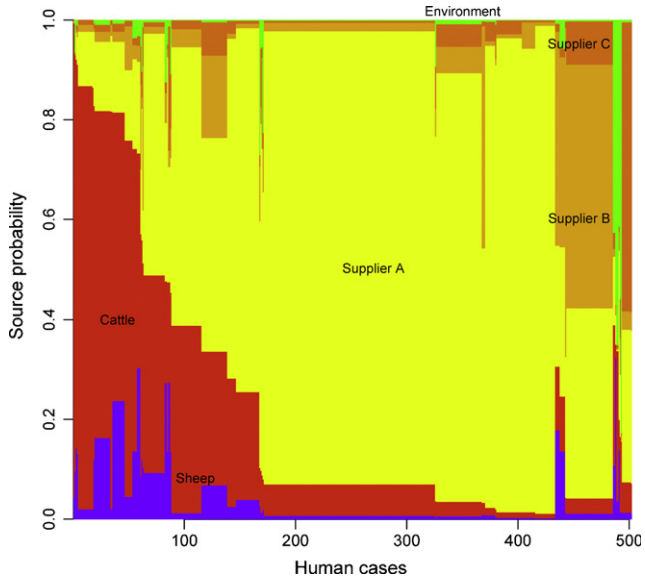

Fig. 1. Source attribution output from asymmetric island model represented as a matrix plot. Each human campylobacteriosis case is a vertical column colored according to the probability it came from each source.

The contribution of the other poultry suppliers as well as ovine and bovine sources was estimated to be similar ranging from 7% to 11%, with cattle estimated to contribute more cases than sheep. The proportion of cases estimated to originate from environmental sources was only 1% (95% CrI 0–6%).

3.4. Asymmetric island model

Poultry was identified by the island model as the most important source of human infection, accounting for an estimated 76% of human cases (Fig. 1). 63% of human cases (95% CrI 55–72%) were attributable to Poultry Supplier A. Suppliers B and C were estimated to contribute only 8% and 4% respectively. The next highest contributor to the burden of human cases was estimated to be ruminants, with cattle estimated to contribute 18% of cases (95% CrI 10–26%).

3.5. Comparing the results from the different approaches

A visual comparison of the output from all three attribution models is shown in Fig. 2. All three models lead to similar

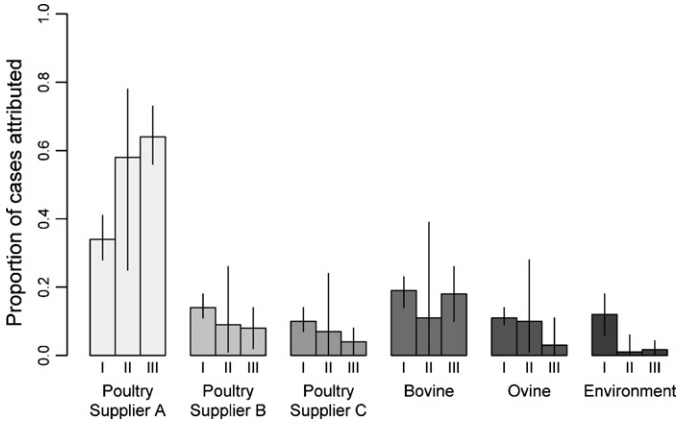

Fig. 2. Proportion of human campylobacteriosis cases attributable to each source: comparing, from left to right, the Dutch (I), modified Hald (II) and asymmetric island model (III). Error bars represent 95% confidence/credible intervals.

estimates, and these are consistent with the PSI. However, the level of precision varied between the individual models. All methods suggest that the majority of cases over the 3-year period could be attributed to poultry, in particular Poultry Supplier A, providing further evidence of the importance of the contribution of this food source to the burden of campylobacteriosis in New Zealand (Eberhart-Phillips et al., 1997). The next most important source was cattle, followed by sheep, with relatively minor contributions from the environment.

4. Discussion

Estimates of the relative contribution of individual sources to human infection can help public health policy makers decide upon the most appropriate control measures to implement. However, for zoonotic pathogens, such as *C. jejuni*, identifying the primary animal reservoirs and transmission pathways is complex and challenging, particularly when these include both domestic and wild animal hosts and a background of changing agricultural and food production practices. The integration of molecular tools into epidemiological approaches to identify the origin of disease has provided relatively high resolution estimates of the importance of different sources to the disease burden of human campylobacteriosis in New Zealand. Our findings support the hypothesis that poultry (Baker et al., 2006), rather than ruminants, wildlife and water (Savill et al., 2001; Devane et al., 2005; Nelson and Harris, 2006; Gilpin et al., 2008b), was the predominant source of human infection between 2005 and 2008.

Here we show that an estimated 58–76% of human cases were attributed to poultry sources, and differences in the contribution from the individual poultry suppliers were observed. These results support previous evidence by microbiological and epidemiological studies (Wagenaar et al., 2006; Gormley et al., 2008; Sheppard et al., 2009) that poultry is the major contributor to the disease burden in industrialized countries. The large variation in the estimated contribution of individual poultry suppliers to the burden of human disease was a surprising observation. Differences in contamination levels of poultry have been found in a recent study in Belgium (Habib et al., 2008), but to date no other study has investigated differences in risk from individual domestic suppliers by considering them as separate sources of infection. The ability to differentiate the contribution of individual suppliers was aided by the relatively simple structure of the New Zealand poultry industry, and could facilitate targeted surveillance and intervention in the future. New Zealand's poultry supply is different to many developed countries in that suppliers are almost entirely focused on the domestic market and for biosecurity reasons no raw poultry products are imported into the country. Due to the geographical isolation and tight border controls the country's poultry industry has to date remained free of diseases endemic in other countries such as certain *Salmonella* (Enteritidis PT4, Typhimurium DT 104) or Newcastle Disease. The sale of fresh poultry increased markedly between 1992 and 2005, whereas the sale of frozen poultry remained relatively static, and it has been suggested that this may have been an important contributor to the increasing incidence of campylobacteriosis over the same time-period (Baker et al., 2006).

The dominance of Poultry Supplier A as infection source was supported by all models. A major contributor to the high number of cases attributed to this supplier is likely to be the high frequency of a particular sequence type, namely ST-474. This sequence type accounted for 30.7% of human cases in our study and was predominantly found in samples from this supplier (French and Molecular Epidemiology and Veterinary Public Health Group Hopkirk Institute, 2008). The production of poultry meat in New Zealand is highly integrated; only three companies supply 90% of

chicken meat, which represents 95% of poultry meat consumed. These suppliers own or control most stages of production, processing and distribution and this is likely to contribute to the observed differences in the occurrence of STs between the different suppliers, with each supplier providing an almost separate niche for bacterial transmission and evolution.

New Zealand provides a distinctive island ecosystem (Crump et al., 2001) and this is evident in the findings from this study. The distribution of *C. jejuni* MLST genotypes in New Zealand is very different to that reported in other countries. For example, internationally rare STs such as the poultry-associated ST-474 and the ruminant-associated ST-2026 are widely distributed in New Zealand (McTavish et al., 2008), and yet common subtypes such as ST-45 and ST-48 are also among the most frequently STs occurring in this ecosystem. The most common ST isolated from environmental water was ST-2381 – this genotype has not been identified anywhere other than in New Zealand water (Carter et al., 2009).

It is striking that the majority of human cases were caused by an internationally rare ST, ST-474, which was also the most commonly isolated genotype in the winter epidemic in 2006 in New Zealand (McTavish et al., 2008). To date this ST has only one submission in the *Campylobacter* PubMLST database (Dingle et al., 2005) from a chicken sample in the Czech Republic and was, other than in New Zealand (French and Molecular Epidemiology and Veterinary Public Health Group Hopkirk Institute, 2008; Taboada et al., 2008), only reported sporadically (Clark et al., 2005; Best et al., 2007). The dominance of this internationally rare clone may be one of the major drivers of New Zealand's relatively high notification rate.

In identifying sources of human campylobacteriosis we chose to model the following: bovine, ovine, poultry (divided into the major suppliers) and environment. However, cases assigned to a source might be acquired by a variety of pathways such as retail meats, occupational exposure and environmental fecal contamination. Hence this framework cannot ascertain the contribution of particular transmission pathways, but attempts to estimate the relative contributions from amplifying animal hosts. We included wild bird and water samples to assess the contribution of potential environmental pathways to the disease burden (French et al., 2008). However, including the environment in these types of models is problematic. Cases from the non-wildlife sources may also be acquired via environmental pathways (such as run-off from dairy or poultry farms). We found a distinctive and diverse distribution of genotypes in our environmental water samples, containing many previously unreported MLST profiles. Their allelic profiles indicate that these are likely to have originated in so far unidentified wildlife sources that are contributing to the presence of *Campylobacter* spp. in environmental water, but are seldom identified in human cases (French and Molecular Epidemiology and Veterinary Public Health Group Hopkirk Institute, 2008; Mullner et al., 2009). Therefore, although environmental water cannot be considered a single amplifying host, it represents a vehicle that delivers a unique exposure primarily from putative wildlife sources.

Bovine sources were estimated to contribute 11–18% of human cases in this study. Given the relatively low prevalence and pathogen counts in ruminant meat samples (Wong et al., 2007), bovine-derived cases are commonly assumed to be the result of direct environmental and occupational, rather than food-borne exposures (Strachan et al., 2009). Occupational exposure includes transmission pathways such as the handling and treatment of animals on farm, as well as the exposure of meat workers to fecal matter during slaughter (Gilpin et al., 2008a). This is in contrast with poultry sources where the dominant transmission pathways are believed to be the handling and consumption of meat (Rosenquist et al., 2003). In addition to changes in food production practices in the poultry industry, pastoral land use in New Zealand has changed markedly over the last two decades, with sheep numbers decreasing by 41% between 1992 and 2008 and dairy cattle numbers increasing over the same period by 61%. Therefore a combination of environmental, occupational and food safety interventions is necessary to reduce the number of cases from sources with multiple transmission pathways. Such complexity of transmission pathways has also been recognized for other zoonoses, such as *E. coli* O157 (Strachan et al., 2006). In consequence interventions for bovine associated campylobacteriosis might include increasing awareness of hygiene when handling livestock or implementing measures to reduce fecal contamination of the environment (Kemp et al., 2005). These findings highlight the importance of combining attribution approaches with detailed studies of the epidemiology and ecology of disease, including aspects such as the spatial distribution of cases, age-related risk factors, pathogen survival in the environment (Ross and Donnison, 2006; Gilpin et al., 2009) and ecological factors contributing to emergence (Woolhouse, 2002; Strachan et al., 2009).

In the multi-model approach applied in this study all models provided broadly similar estimates of the contribution of each source to the burden of human infection. Further, the results are in general agreement with the findings of earlier studies conducted in New Zealand (Eberhart-Phillips et al., 1997) and elsewhere (Pearson, 2000). There are some notable exceptions; for example the estimated contribution from environmental sources varies from 1% (modified Hald model) to 2% (asymmetric island model) to 12% (Dutch model). The divergence of the estimate from the Dutch model might be explained by the model's assumption of equal impact between the sources, which results in more evenly distributed attribution estimates and thereby in an overestimate of the contribution of environmental water sources. This is also supported by the low PSI between human and environmental sources.

In its application the PSI is the most straightforward measure to calculate, and possibly the easiest to interpret. Although this technique does not estimate the number or proportion of human cases attributable to each source, it may be considered a useful first-step in the assessment of source attribution, by providing a measure of the correlation between source distributions (Garrett et al., 2007). The Dutch model assumes that all strains are considered to have an equal probability of causing human disease, at a given dose in a given source, and all sources contaminated with *C. jejuni* are assumed to have an equal probability of causing disease in humans. However, it is highly likely that some STs are more virulent or pathogenic than others (Pope et al., 2007), and therefore more likely to cause human disease at a lower dose or to cause more severe disease. Further, some food sources may be considered to be more 'risky' than others due to variation in, for example, the level of contamination, properties of the food matrix and the effects of cooking on thermal inactivation, as some foods are more likely to be undercooked than others (Uyttendaele et al., 1999; Cornelius et al., 2005). Since the Dutch model assumes equal impact between sources, the number of cases tends to be more equally distributed among sources as compared to the other models.

In contrast to the Dutch model, the modified Hald model does explicitly consider both bacteria and food-type dependent factors, and incorporates both sampling and parameter uncertainty using Bayesian inference. The estimates from our modified Hald model are associated with large uncertainty. This lack of precision is partially overcome when pooling individual poultry suppliers into one group (Mullner et al., 2009), and is the result of decreased heterogeneity of genotypes between sources when poultry suppliers are modeled individually.

By taking a population-genetics approach, the asymmetric island model (Wilson et al., 2008) is substantially different from the above models. The relatedness of genotypes is considered explicitly, and all human isolates can probabilistically be assigned to individual sources. The credible intervals for the island model are narrower than for the modified Hald model, most likely because more information (the allelic profiles), is used to fit the model.

In this study, a 3-year structured longitudinal study, integrating human, food and domestic and wild animal surveillance data, allowed us to apportion cases of disease to a source, which is in contrast to the cross-sectional surveys, using data from different geographical regions and time-periods, reported in other studies (McCarthy et al., 2007a,b; Wilson et al., 2008; Strachan et al., 2009). In our sentinel site study 2766 samples were collected over a 3-year period. By simultaneously collecting data from human clinical cases and potential disease sources in a defined region we aimed to capture human exposure over an extended period. A random sample of a total of 969 samples were typed (80% of all *C. jejuni* isolates), consisting a subset of *C. jejuni* isolated in the sentinel surveillance site during the study period. These isolates were selected to be representative of each source and each month of the study. Comparative studies conducted in two other regions show a similar distribution of genotypes in both humans and poultry (French and Molecular Epidemiology and Veterinary Public Health Group Hopkirk Institute, 2008). This suggests that the results from the study site are likely to be similar to that in other regions and allow for extrapolation to the whole of New Zealand.

Our attribution estimates can be compared with those of a study conducted in Lancashire, England (Wilson et al., 2008), the major difference being the relatively higher contribution from cattle (35% in Lancashire, 11–19% in the Manawatu) and a lower contribution from poultry sources (57% in Lancashire, 58–76% in the Manawatu). This may be explained by a higher relative consumption of poultry meat in New Zealand and the presence of unique poultry-associated genotypes (Baker et al., 2006). However, estimates from our study are very similar to those from a study conducted in Scotland (Sheppard et al., 2009), where poultry sources were associated with 58–78% of infections. The comparatively high number of cases associated with poultry was an unexpected finding for New Zealand, as it was previously hypothesized that environmental sources may be an important contributor to the disease burden in this country (Savill et al., 2001) and this may also explain the high notification rate. However, this study has provided evidence that the relative contributions from different source are similar to other countries, although the *Campylobacter* STs detected are markedly different. This is an interesting finding and gives new insight into the emergence of this pathogen, which in New Zealand may have been driven by multiple introductions from multiple sources.

The estimated low importance of non-livestock sources estimated by these recent studies contrasts with the finding by Champion et al. (2005). Using a whole genome micro-array, they concluded that non-livestock, non-agricultural sources may be the most important for human infection. The striking difference in the conclusion may be attributed to the selection of isolates for inclusion in the analysis and model specification (Wilson et al., 2008).

The evidence provided by our approach has supported national policy making by providing an important contribution to the New Zealand Food Safety Authority (NZFSA) *Campylobacter* Risk Management Strategy (2007), which has subsequently included mandatory targets for limiting contamination with *Campylobacter* spp. of chilled poultry carcasses. The introduction of these interventions has coincided with a dramatic decrease in human campylobacteriosis notifications to a 16-year low. In 2008 some 6689 human cases were reported in New Zealand compared to 15,873 cases in 2006; the year before the announcement and implementation of control measures. A logical next step is to monitor changes in source attribution post-intervention to ascertain whether the relative importance of poultry has diminished. In addition further refinement of the sampling scheme and source attribution models should enable finer-scale temporal changes in attribution to be described. This will be valuable for determining seasonal changes in the relative contribution of sources (Nylen et al., 2002) and provide a dynamic tool for assessing the effectiveness of interventions.

In conclusion, a combination of epidemiology, ecology, molecular biology and population-genetics has provided a valuable risk-based approach to inform surveillance and public health policy for the control of enteric zoonotic pathogens. Given the use of temporally and spatially appropriate sampling strategies these tools have the potential to be applied to inform decision making for the control of other zoonoses, such as cryptosporidiosis and salmonellosis. Furthermore, the risk attribution framework presented here can be modified to enable continuous, dynamic assessment of source attribution, enabling seasonal variation in infection sources to be identified, and facilitate an assessment of the effectiveness of control programs.

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