Elucidation of potential transmission routes of
Campylobacter in New Zealand

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Abstract Campylobacter is the most commonly reported notifiable disease in New Zealand. The cost of Campylobacter infections in the country during 1994 was estimated as $61.7M although the true cost was probably higher. Investigation of the main environmental reservoirs and routes of transmission to humans is necessary to formulate the most appropriate intervention strategies. This project investigated the reservoirs of Campylobacter in a defined geographical area within New Zealand and compared strains isolated from humans and environmental sources within this area as a prelude to investigating the likely transmission routes to humans. Campylobacter jejuni was commonly found in faeces from dairy cows, beef cattle, sheep and ducks, chicken carcasses, sheep offal and surface waters and C. coli was commonly found in sheep faeces. Preliminary analysis of Penner types was suggestive of transmission to humans from dairy and beef cattle and possibly from sheep.

Keywords Animal faeces; Campylobacter; food; transmission routes

Introduction Campylobacteriosis is by far the most frequently notified disease in New Zealand (NZ) with an annual rate of 233 cases/100,000 (Lopez et al., 2001). It has about 5× the rate of salmonellosis and its notified incidence in New Zealand is more than 3× higher than the rate reported by other developed countries (Lopez et al., 2001). It is well known that chicken products are frequently contaminated by this organism (Campbell and Gilbert, 1995) and a study (known as the Multi-centre Analysis of Gastro-enteritis Induced by Campylobacter, the MAGIC study) identified some aspects of chicken consumption as risk factors (Eberhardt-Phillips et al., 1997). It has been stated that risk factors from barbequed meat and undercooked poultry, drinking water and travelling abroad have been indicated in only 5–15% of campylobacteriosis cases (Nielson and Nielson, 1999). This leads to the conclusion that there is a large gap in our knowledge of the other sources of Campylobacter. Campylobacter jejuni and C. coli cause >95% of all campylobacteriosis infections (Koenraad et al., 1997). In order to understand the epidemiology of Campylobacter in NZ, it is necessary to identify the main reservoirs of these two Campylobacter species that impact on human health.

The intestinal tract of many warm-blooded animals has been identified as a major reservoir for Campylobacter species (Koenraad et al., 1997). From New Zealand and overseas studies there is evidence that Campylobacter is frequently present in monogastric and ruminant animals (Kakoyiannis et al., 1988; Meanger et al., 1989). Bird populations, including blackbirds and gulls, have been associated with outbreaks of campylobacteriosis (Stuart et al., 1997). From these observations it seemed likely that farm animals and bird populations may be acting as significant reservoirs of Campylobacter and serve as contamination sources of farm water run-off, for farm workers, their families and for meat processing workers. In this study we sought to identify the frequency of the two major human pathogenic species and the relationship of the individual types of Campylobacter isolated from animal faeces to the other reservoirs and transmission routes.
A New Zealand study (Kakoyiannis et al., 1988) reported that 49.7% of the human isolates typed were indistinguishable from poultry isolates. More recent research (Koenraad et al., 1995; Korolik et al., 1995) has also found strains of Campylobacter that were unique to poultry and were not found in the other matrices tested to date. In overseas studies it has been reported that Campylobacter was present in raw chicken portions. The carriage rate in chickens varied from 77% in a United Kingdom study (Kramer et al., 2000), 60% in a USA study (Konkel et al., 1999) and 46% in a Japanese study (Ono and Yamamoto, 1999). Other studies have isolated Campylobacter from raw meat products (Weijtens, 1999), but Ono and Yamamoto (1999) failed to detect C. jejuni in beef and pork. Offal was chosen as the source of red meat for this project because it more commonly contains Campylobacter than other meat cuts (Kramer et al., 2000). It is, therefore, possible that these foods (lamb, mutton, beef and pork) act as significant reservoirs of infection.

The MAGIC study identified drinking water that was supplied from sources other than town supply, e.g. rainwater, as a risk factor of campylobacteriosis (Eberhardt-Phillips et al., 1997). In a recent survey carried out jointly by the Ministries of Health, Environment & Agriculture and Forestry, Campylobacter was detected at concentrations >1/mL on occasions in a number of surface waters throughout the country (Savill et al., 2000). Furthermore, PCR analysis identified that many of the water samples contained multiple species of Campylobacter. The present project analysed surface waters for the species and strains of Campylobacter to examine the flow of pathogen contamination within the study area.

Whilst clinical specimens are routinely analysed using conventional culture methods, isolates are rarely identified to species level and when identified only one species is reported, generally C. jejuni. To understand the epidemiology of Campylobacter infections in NZ, it is necessary to identify the clinical isolates to at least species level and to relate the types present to environmental sources. Speciation, typing and sub-typing of Campylobacter isolates are necessary for establishing linkages between environmental and human sources of infection, tracking outbreaks and potential changes in disease aetiology.

Serotyping (a phenotypic method) is an important preliminary screen of strains. Molecular sub-typing techniques (also referred to as genotyping) are now recognised as the

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**Figure 1** Potential reservoirs and transmission routes for Campylobacter
most discriminatory techniques for investigating the routes of transmission from animals to humans (Wassenaar and Newell, 2000), allowing strain relationships between isolates from differing geographical locations to be ascertained. To maximise the value of the data, there is a requirement for the ability to share data between laboratories. Such a data sharing capability is made possible by specialised software packages (e.g. the US PulseNet) that allow normalisation of data to a recognised standard reference. PulseNet is a national public health resource that facilitates the rapid tracking of disease outbreaks through the use of molecular sub-typing. This computer-based network has primarily been applied to food-borne disease surveillance. Figure 1 gives an overview of the transmission routes which could be involved in the increase of campylobacteriosis.

Methods
During the 12-month study during 2001, a survey was conducted of animals, foods produced from them, waters potentially polluted by the animals and infected humans within the study area. Samples comprised (a) composite faeces from farmed animals (275) and birds (92), (b) river waters (300), (c) sheep, pork and beef offal (527), (d) chicken carcasses (200), (e) human faecal samples (78) and (f) individual faecal samples from rabbits (99) and possums (260). All samples were enriched in Exeter broth using two sequential selective enrichments. The first enrichment was for 4 h at 37°C followed by 44 h at 42°C. A second enrichment for 24 h at 42°C was to ensure that the subsequent PCR detection was of viable cells and did not detect dead cells that may have been present in the samples. Positive, negative and sterility controls were included to ensure that contamination of the broth media had not occurred and that selective enrichment of Campylobacter was occurring. DNA was extracted from the enrichment broth and subjected to PCR (Figure 2).

DNA/PCR methods were developed and validated for meat, offal (sheep, beef, pork), water and faecal (human, cattle, sheep, dairy, duck) samples, allowing multiple species identification (C. jejuni, C. coli) from a single sample (manuscript in preparation).

The relationship between individual isolates of C. jejuni and C. coli were determined by a combination of Penner serotyping (Penner and Hennessy, 1980) and pulsed field gel electrophoresis (PFGE) (Gibson et al., 1994). These approaches have been found to be reproducible, robust and provided results that could be exchanged between laboratories (Nicol and Wright, 1998). Pure cultures were isolated from PCR-positive samples and analysed using Penner and/or PFGE typing. Each PFGE gel included three evenly spaced standard lanes consisting of the lambda concatemer from New England Biolabs. These sub-typing...
methods have been used internationally, are widely accepted and will promote information exchange with overseas agencies. Our aim was to generate results that could be collated onto a NZ/International database so that *Campylobacter* transmission could be effectively monitored nationally and globally. To achieve this outcome we have entered all data into the Bionumerics software (the database software of choice for Campynet in Europe and Pulsenet in the USA).

The exposure of human cases exposed to various risk factors was investigated using the *Campylobacter* Questionnaire administered by NZ Crown Public Health. Additional questions were added to the routine Questionnaire addressing specific concerns of this project.

**Results**

About 630 samples were positive for *Campylobacter* (Table 1).

Approximately 25 Penner and 70 PFGE groups were observed. *Campylobacter* was more common in farmed animals than other matrices, in general, but was less common or absent in the feral animals studied. The incidence of *C. coli* at 47% was higher in sheep faeces than expected although this was not reflected in sheep offal. However, the incidence of *C. jejuni* in chicken was lower than expected (26%). Food products contained *Campylobacter* less frequently than water or farmed animal faeces. More types were identified in water than in any other matrix.

The serotype groupings in three of the major matrices are shown in Figure 3. Water, human and dairy faeces were chosen since they all had significant numbers of samples that contained *Campylobacter* and appeared to show some relationships or have common grouping. When the major serotypes identified in the human samples were examined and compared with those from environmental samples, the following was observed:

(i) the major Penner types in humans that occurred in other matrices and possibly showed temporal linkages were 1,4,4; 2; 23,36,4; and smaller groups of 6; 57; 10 and 19;

(ii) three of these types 2, 4 and 23,36 were significant groups in another study that investigated the presence of particular *C. jejuni* types in dairy cows and humans in another region of NZ (manuscript in preparation);

(iii) these eight Penner types suggested possible temporal transmission pathways: dairy faecal material/cattle faecal material to water to humans; dairy/cattle faecal material to humans; sheep to humans; chicken to humans and pigs to humans;

(iv) sheep faecal material carried the same types of *Campylobacter* as the other matrices and could well be involved in the transmission. Further spatial and PFGE analysis of the data is required;

(v) some of the same common types in humans, water and dairy cattle were found in ducks.

<table>
<thead>
<tr>
<th>Sample type</th>
<th>% with <em>C. jejuni</em></th>
<th>% with <em>C. coli</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Dairy cow faeces (composite from 5 animals)</td>
<td>96</td>
<td>10</td>
</tr>
<tr>
<td>Beef cattle faeces (composite from 5 animals)</td>
<td>85</td>
<td>12</td>
</tr>
<tr>
<td>Sheep faeces (composite from 5 animals)</td>
<td>56</td>
<td>47</td>
</tr>
<tr>
<td>Rabbit faeces</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Possum faeces</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Duck faeces (composite from 5 birds)</td>
<td>65</td>
<td>2</td>
</tr>
<tr>
<td>Surface water</td>
<td>51</td>
<td>2</td>
</tr>
<tr>
<td>Chicken carcasses</td>
<td>26</td>
<td>1</td>
</tr>
<tr>
<td>Sheep offal</td>
<td>37</td>
<td>3</td>
</tr>
<tr>
<td>Beef offal</td>
<td>9</td>
<td>2</td>
</tr>
<tr>
<td>Pork offal</td>
<td>5</td>
<td>4</td>
</tr>
</tbody>
</table>

*Table 1* Summary of *Campylobacter* isolations
Conclusions

The collection of environmental samples and their analysis for species and types of Campylobacter proved to be useful in identifying likely transmission routes of Campylobacter in New Zealand. Since the country has an agriculturally based economy, high animal loadings are carried with over half the land area devoted to agriculture (45 million sheep and nine million cattle). This is estimated to produce $45 \times 10^{12}$ tonnes of faecal material passing into natural waterways (Murray and Savill, 2000). The results of this project are currently being analysed for PFGE patterns and statistical interpretation of these findings. These additional results will help clarify the first Penner findings discussed here. Even with the first Penner results a potential role of animal faecal samples transmitting Campylobacter to the human population is being detected, either directly or via surface water. Additional transmission pathways of food (particularly chicken and sheep) to humans were also suggested. If these results are subsequently confirmed then either intervention steps can be reviewed or particular transmission routes examined in more detail.

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