

Prevalence of *Campylobacter* spp. in diarrhoea samples from patients in New South Wales, Australia

Aruna Devi,¹ Adam S. Gunjilac,² Jenny M. Wilkinson,¹ Thiru Vanniasinkam,¹ Timothy J. Mahony^{2,*}

¹School of Biomedical Sciences, Charles Sturt University, Wagga Wagga, Australia.

²Queensland Alliance for Agriculture and Food Innovation, University of Queensland, Brisbane, Australia

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Summary. Campylobacteriosis is a leading cause of bacterial foodborne disease in many industrialized countries including Australia. New South Wales (NSW) is the most populous state in Australia yet the lack of any *Campylobacter* species surveillance programs has led to a knowledge gap in the importance of these pathogens as causes of diarrhoea. The data collected in this study demonstrated a need for such programs. In this study, 400 human clinical fecal samples were collected from two NSW locations, Western Sydney and Wagga Wagga, and tested for the presence of *Campylobacter* spp. Patients were clustered by location, age and gender to assess *Campylobacter* spp. prevalence within these groups between the two regions. The frequency of *Campylobacter* spp. was higher in males compared to females in the age groups 0–4 and 5–14 years; 6.4% and 1.0%, and 8.2% and none, respectively ($P < 0.05$). A second peak was noted in elderly adults compared with those in younger age groups. Based on the findings of the quantitative PCR analysis it was estimated that the age-adjusted prevalence of *Campylobacter* spp. associated diarrhoea was 159 cases per 100,000 persons. [Int Microbiol 2016; 19(1):33-37]

Keywords: *Campylobacter* species · campylobacteriosis · foodborne diseases · prevalence of pathogens · New South Wales, Australia

Introduction

In Australia, approximately 5.4 million cases of foodborne diseases are reported annually resulting in an estimated cost to the health system of \$1.2 billion per year. *Campylobacter* species have been reported to be the most common enteric patho-

gens amongst all known foodborne pathogens in Australia. The annual report produced by “OzFood Net” [21], an Australian government initiative which reports on the incidence of foodborne diseases, reported 16,968 cases of *Campylobacter* spp. In 2010. This report, however, excluded the most populous state, New South Wales (NSW) [13].

Campylobacteriosis is the leading cause of bacterial foodborne disease in industrialized countries, including Australia. The most prevalent species seen in human infection are *Campylobacter jejuni* and *C. coli* [15]. Human campylobacteriosis is generally a self-limiting disease with typical enteric symptoms including vomiting, fever, diarrhoea and abdominal pain [13,25]. Chronic diseases such as reactive arthritis and Guillain-Barré syndrome may occur sporadically as a

*Corresponding author: T.J. Mahony
Queensland Alliance for Agriculture and Food Innovation
Queensland Bioscience Precinct
University of Queensland
306 Carmody Road
Brisbane, QLD 4072 Australia
Tel. +61-733466505. Fax +61-733466555
E-mail: t.mahony@uq.edu.au

consequence of campylobacteriosis [19]. *Campylobacter* spp. infections are widely prevalent in Australia and infection rates have shown a steady increase in recent years [13]. Most campylobacteriosis cases in Australia are thought to be sporadically acquired [29], showing a similar infection pattern to studies conducted in North America [8,23], Europe [10,12] and New Zealand [26].

The most important route of *Campylobacter* infection is the consumption and handling of poultry [3]. In fact, in Australia, the United States and Europe, 50–70% of all *Campylobacter* spp. infections have been attributed to the consumption of undercooked or contaminated chicken [1,18]. Furthermore, improper food handling or inadequate hygiene techniques, as well as household pets and domestic chickens are thought to be linked to campylobacteriosis in children and adults [28].

However, there has been no direct investigation of the wide range of potential human exposure sources or systematic exploration of possible ecological cycles of *Campylobacter* spp. in Australia. Human *Campylobacter* spp. infection is a communicable disease in all Australian states and territories, apart from NSW, and the data collected can be used to inform prevention and control measures. The exclusion of NSW from this system means there is no accurate information on either the prevalence or incidence of *Campylobacter* spp. infections in this state, which makes the development of more effective control policies and measures problematic [13].

The aim of this study was to estimate the prevalence of *Campylobacter* spp. in patients with symptoms of diarrhoea living in two areas of NSW who sought medical care. Prevalence of *Campylobacter* spp. in different study population categories were also compared to determine if factors such as patient age or gender influenced the *Campylobacter* spp. infection prevalence.

Materials and methods

Study design and locations. A cross-sectional study was conducted to investigate the prevalence of *Campylobacter* spp. infection at the Microbiology Department of the Westmead Hospital in Western Sydney and the Microbiology Department of the Wagga Wagga Base Hospital from October 2012 to February 2014. A total of 400 ($n = 200$ Wagga Wagga, $n = 200$ Western Sydney) diarrhoeal samples, as defined according to World Health Organization guidelines, were opportunistically collected from the faecal samples submitted for routine analysis and culture at these two laboratories for the duration of the sampling period. The Australian Bureau of Statistics has described Sydney and Wagga Wagga as a major city and an inner regional city respectively.

Sample collection. Clinical faecal samples were aliquoted in sterile containers and an appropriate code was assigned to each sample. The samples

were refrigerated after routine testing was completed by the Microbiology Departments at both locations. Samples from western Sydney were placed on ice and transported to Charles Sturt University for DNA extraction within 24 h of collection. Samples collected at the Wagga Wagga Base Hospital were transported to the university laboratory within one hour and processed immediately.

DNA extraction. Total DNA was extracted from human faecal samples using QIAamp DNA Stool Mini Kit (QIAGEN Pty Ltd, Australia). An aliquot of the sample (200 μ l) was transferred to a 2-ml Eppendorf tube and heated to 70 °C for 10 min. Total DNA was extracted using the manufacturer's protocol. The final elution volume of DNA was 200 μ L. The DNA samples were stored at –20 °C prior to PCR analysis.

Real time PCR assay (qPCR). The presence or absence of DNA from *Campylobacter* spp. in the clinical sample extracts was determined by using quantitative real-time PCR (qPCR). The qPCR primers and the probe used were modified from previously published studies [4,6]. The qPCR assay was designed to detect all *Campylobacter* spp. by targeting the 16S rRNA gene using the primer pair Lund_16S_F 5'-CGT GCT ACA ATG GCA TAT ACA ATG A-3' and Lund_16S_R 5'-CGA TTC CGG CTT CAT GCT C-3'. A novel 16S probe, Campy_16S-LNACy5 *Campylobacter* Cy5-5'-ATA [+G] AT [+T] TC [+C] AC C-3'-BHQ3 (Sigma-Aldrich, Australia) was designed using Beacon Designer version 8.12 (Premier Biosoft International). The nucleotide residues designated as [+N] have been modified with locked nucleic acid technology [5,14]. Briefly, the LNA probe was designed by importing the forward and reverse oligonucleotides into the software, and the default settings used to identify the optimal probe sequence within the 44bp amplicon. To confirm that the optimal probe sequence was identical to *Campylobacter* spp. 16S sequences and to assess the likelihood of cross-reaction with bacterial species that might be present in clinical samples, a Primer-BLAST search was performed [30].

All qPCR assay runs included control templates from strains; *C. jejuni* NCTC 11168, *C. jejuni* ATCC 29428, *C. jejuni* ATCC 49943, *C. coli* NCTC 11366, and *C. coli* ATCC 33559. Each qPCR reaction volume of 20 μ l contained 10 μ l of 2 \times TaqMan master mix (Applied Biosystems), 16S forward primer 0.2 μ M, 16S reverse primer 0.2 μ M, 16S probe 0.2 μ M. DNA template 1 μ l was used with the following conditions; Taq Hot Start for 15 min at 95 °C, one cycle, 40 cycles of denaturation for 1 min at 95 °C, annealing and extension for 1 min at 60 °C in the Rotor-Gene Q (QIAGEN).

Data analysis. The qPCR results were analyzed using Rotor-Gene Q Series software. The cycle threshold values determined based on the run parameters with software default setting. Population denominators were obtained for the relevant NSW regions from the Australian Bureau of Statistics data, with 2011 being the most recent data collection year. The Western Sydney population for the year 2011 was 846,001 and for Wagga Wagga was 237,338. For the estimation of prevalence per 100,000, the NSW 2011 standard population data of 7,218,529 was used [20,22]. Based on the age distribution of cases used in previous studies [7,9], the following age groups were identified for data analysis and age-specific prevalence of *Campylobacter* spp.: 0–4, 5–14, 15–24, 25–34, 35–44, 45–54, 55–64, 65–74 and ≥ 75 years were calculated. Samples from all age groups were included in this study. Samples from both genders were also included in the study.

The sample results were tabulated using the package software Statistical Package for the Social Sciences (SPSS, version 20). An unconditional logistic regression model was used in multivariate analysis. Chi-square analysis was performed to compare results in various groups with $P < 0.05$, deemed to be statistically significant.

Ethics approval. This study was approved by the Charles Sturt University Ethics in Human Research Committee, with approval for sample collec-

tion also granted by the Pathology Department managers at each of the hospital sites used in this study. The approvals permitted the collection of basic patient information on age and gender, in a de-identified manner to protect patient confidentiality.

Results and Discussion

Prevalence of infection. This study reports a *Campylobacter* spp. population adjusted prevalence of *Campylobacter* spp of 159 cases per 100,000 population in NSW (Fig. 1). The estimate was determined by testing, in diarrhoea samples (n = 400) collected from the two locations, the presence of *Campylobacter* spp DNA by qPCR. *Campylobacter* spp. were detected in 209 of the samples tested, giving a *Campylobacter* spp. prevalence of 52.3% (95%; confidence interval 47.8–58.3%) (Table 1). The NSW prevalence estimate was higher than the Australian case rate of 93.5 cases per 100,000 population, without including NSW data during 2013 when the majority of the study samples were collected [2]. Regarding the various Australian states, the data collected in this study suggested that NSW and Tasmania the most similar rates of human *Campylobacter* spp. infections, with 158.9 and 135.7 cases per 100,000 population respectively for 2013 (Fig. 1). Somewhat surprisingly, the Australian Capital Territory, located within NSW, had a much lower case rate of 98.4 cases per 100,000 for this year compared to the jurisdictions (Fig. 1) [2]. Differences in population size, demographics and health polices within each juris-

diction suggest that additional research is required to establish if risk factors for infection are also similar.

The higher prevalence of *Campylobacter* spp. in NSW reported in this study compared to other states may have resulted from the detection methodologies used. Culture is often considered the gold standard technique for *Campylobacter* spp. and it is used in most microbiology laboratories across Australia, while the current study has used qPCR to determine the results for NSW. It is well established in the literature that qPCR is more sensitive than culture for the detection of fastidious [16,17,24]. Due to the fastidious and liable nature of *Campylobacter* spp., culture-based detection might underestimate the true prevalence in a population. In contrast, qPCR would be more likely to overestimate the true prevalence of *Campylobacter* spp. as it could detect both live and dead bacteria in the samples. This study utilised qPCR as the detection method to ensure that the results from samples collected at different locations were comparable. In fact, samples from Western Sydney could not be tested for at least 24 h after collection while those from Wagga Wagga could be processed just one hour after having been collected. Therefore, the use of culture could have dramatically underestimated the true prevalence in Western Sydney. Moreover, the estimated prevalence for NSW in this study is consistent with, albeit higher, analogous data from other Australian states determined using culture (Fig. 1), which suggests that qPCR detection is a rapid, sensitive and cost-effective method of detecting this pathogen.

Fig. 1. Comparative prevalence of *Campylobacter* species infection per 100,000 population in NSW (this study) and other Australian states and territories in 2013; Australian Capital Territory (ACT), New South Wales (NSW), Northern Territory (NT), Queensland, (Qld), South Australia (SA), Tasmania (Tas), Victoria, (Vic), and Western Australia (WA). The 2011 population census NSW HealthStats from the Australia Bureau of Statistics were used to calculate rate per 100,000 population.

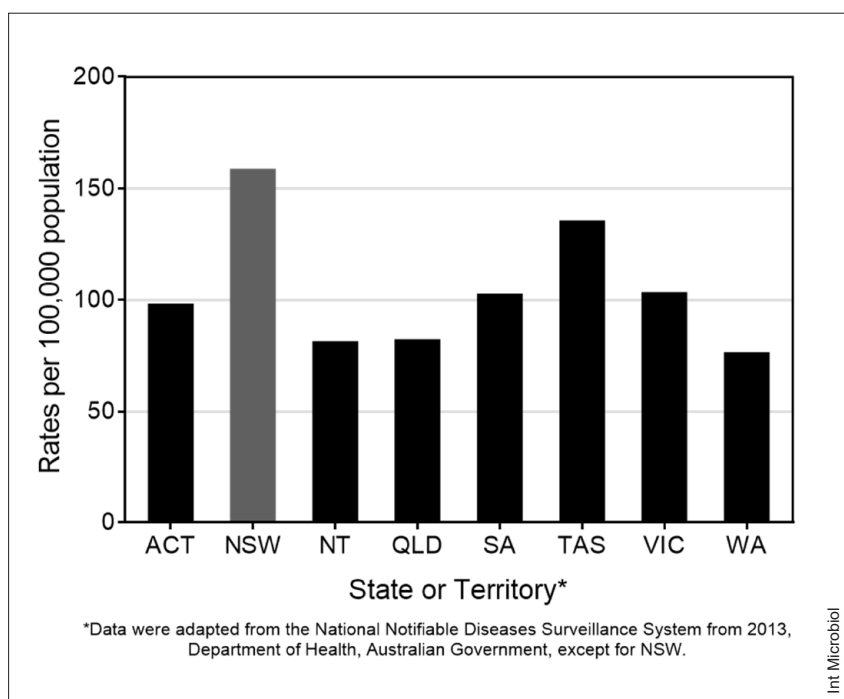


Table 1. Categorisation of *Campylobacter* spp. positive diarrhoeal specimens by age and gender determined using quantitative real time PCR (n = 394*)

Age group (years)	All Samples		Patient sex	
	Tested	Positive (%)	Male Positive (%)	Female Positive (%)
0-4	12	8 (3.8)	7 (6.4)	1 (1.0)
5-14	15	9 (4.3)	9 (8.2)	0
15-24	24	10 (4.8)	6 (5.5)	4 (4.0)
25-34	34	20 (9.5)	9 (8.2)	11 (11.1)
35-44	23	16 (7.7)	5 (4.5)	11 (11.1)
45-54	40	20 (9.6)	9 (8.2)	11 (11.1)
55-64	58	35 (16.7)	21 (19.0)	14 (14.1)
65-74	76	41 (19.6)	21 (19.0)	20 (20.2)
>75	112	50 (23.9)	23 (20.9)	27 (27.3)
Total	394*	209	110	99

* Information on patient age and gender for six samples, of which two were positive from Wagga Wagga, were missing and therefore not included in this table.

Distribution of infection by age. The distributions of *Campylobacter* spp. positive samples by age and gender are shown in Table 1. The highest prevalence of *Campylobacter* spp. infections were found in age groups 55–64, 65–74 and ≥75 years (16.7%, 19.6% and 23.9%) respectively. The lowest numbers of cases were in children and youngsters aged 0–4, 5–14 and 25–34 (3.8%, 4.3% and 4.8% respectively) (Table 1). Age details were not available for six samples two of which were positive for *Campylobacter* spp. and as a result these could not be included in the analysis. A previous cohort study reported a higher prevalence for the 0–4-year-old group [11]. In comparison the current study included samples from all age groups as it was reliant on the opportunistic sampling of the study population. Another reason for the lower prevalence and reduced number of samples in the 0–4-year-old group of the current study could be the limited pediatric services provided at the Westmead hospital where the sampling was conducted; therefore, patients in this age group might have been treated in another hospital such as Childrens' hospital for specialised treatment.

Prevalence of infection by gender. When comparing males and females, no significant differences in the *Campylobacter* spp. prevalence were found, although the frequency was slightly higher for males (52.6%) than for females (47.4%) (Table 1). In the age groups 0–4 and 5–14 years, statistically significant differences were noted in the number of *Campylobacter* spp. infection in males (6.4%) compared with females (1.0%), and 8.2% in males

and none in females, respectively ($P < 0.05$). In the 35–44 age range, significantly higher prevalences of *Campylobacter* spp. were detected in females with 11.1% compared to 4.5% in males ($P < 0.05$, Table 1). For the age groups 25–34 and ≥75, the prevalences were higher in females than in males ($P < 0.05$, Table 1). The gender differences in *Campylobacter* spp. prevalence identified in the present study were largely in agreement with rates reported in another similar study [27].

We can conclude that this study provides significant data on selected demographic determinants, as well as trends of *Campylobacter* spp. infections in NSW, Australia. The data collected in this study suggest that NSW might have the highest prevalence of *Campylobacter* spp. infections among all of the Australian health jurisdictions. To obtain more detailed data on the prevalences of *Campylobacter* spp. infections in the whole country, mandatory reporting in NSW would be highly desirable as this would effectively establish a national surveillance program. The results reported in this study suggest that more research into the role of *Campylobacter* spp. in the cases of diarrhoea could help to determine the effects of a wide range of risk factors in NSW and all Australia and underpin better control measures. 🇺🇸

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