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Investigating locally-relevant risk factors for *Campylobacter* infection in Australia: protocol for a case-control study and genomic analysis

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ABSTRACT

Introduction:

The CampySource project aims to identify risk factors for human *Campylobacter* infection in Australia. We will investigate locally-relevant risk factors and those significant in international studies in a case-control study. Case isolates and contemporaneous isolates from food and animal sources will be sequenced to conduct source attribution modelling, and findings will be combined with the case-control study in a source-assigned analysis.

Methods and analysis:

The case-control study will include 1,200 participants (600 cases and 600 controls) across three regions in Australia. Cases will be recruited from campylobacteriosis notifications to health departments. Only those with a pure and viable *Campylobacter* isolate will be eligible for selection to allow for whole genome sequencing of isolates. Controls will be recruited from notified cases of influenza, frequency matched by sex, age group and geographical area of residence. All participants will be interviewed by trained telephone interviewers using a piloted questionnaire.

We will collect *Campylobacter* isolates from retail meats and companion animals (specifically dogs), and all food, animal and human isolates will undergo whole genome sequencing. We will use sequence data to estimate the proportion of human infections that can be attributed to animal and food reservoirs (source attribution modelling), and to identify spatial clusters and temporal trends. Source-assigned analysis of the case-control study data will also be conducted where cases are grouped according to attributed sources.

Ethics and dissemination:

Human and animal ethics have been approved. Genomic data will be published in online archives accompanied by basic metadata. We anticipate several publications to come from this study.

KEYWORDS

Campylobacter, case-control study, risk factors, Australia, whole genome sequencing, source attribution, source-assigned analysis

ARTICLE SUMMARY

Strengths and limitations of this study

- Case-control study is well-powered to identify locally-relevant risk factors.
- Linking genomic data to the case-control study strengthens the analysis by enabling source attribution and source-assigned analyses to be conducted.
- Case-control questionnaire questions are being validated in a separate study, demonstrating the reliability of participant recall.
- Potential reporting bias due to inaccurate recall of study participants.
- Case-control study lacks efficiency for risk factors with high levels of exposure in the study population.

INTRODUCTION

Campylobacter infection is the most commonly notified cause of foodborne gastroenteritis in Australia,[1-3] as well as a leading cause of bacterial gastroenteritis world-wide.[4] At the introduction of Australia's National Notifiable Diseases Surveillance System (NNDSS) in 1991 the incidence rate of notified campylobacteriosis cases was 79.1/100,000 population,[5] and despite notification rates plateauing in recent years, incidence had risen to 139.7/100,000 population in Australia in 2015,[5] with an estimated 10 cases for every notified case within the community.[6] By comparison, the incidence rate of campylobacteriosis in New Zealand in 2014 was 150.3/100,000 population,[7] with an estimated 10-30 cases in the community for every notified case.[8] *Campylobacter* notification rates in Australia and New Zealand are still among the highest in the world across high-income countries. Most countries in the European Union consistently report annual campylobacteriosis notification rates below 100/100,000 population.[2]

Two species of *Campylobacter—Campylobacter jejuni* and *C. coli*—contribute to approximately 95% of human campylobacteriosis.[9] These *Campylobacter* species are commonly detected in sewage and surface water,[10] reside in the gastrointestinal tract of birds and animals,[11] and are frequently found in raw meat, particularly poultry, and raw milk.[12 13] Campylobacteriosis is mostly foodborne, with an estimated 77% of cases transmitted via food consumption in Australia.[14 15] Direct and indirect zoonotic transmission can occur via animal contact (direct) or faecally-contaminated water or environments (indirect). Person-to-person transmission is considered rare.[16] The majority of cases are thought to be sporadic, with outbreaks less commonly detected.[17] Most outbreaks are linked to the consumption of poultry, raw milk, or contaminated water.[17 18]

Targeted control of foodborne bacterial pathogens generally depends on identification of sources and routes of transmission. Since *Campylobacter* are ubiquitous in the environment and most cases are sporadic, identifying sources is difficult. Source attribution methods require isolation of strains from reservoirs to compare *Campylobacter* strain diversity in foods and animals to that in human infections. Beef, sheep and pig meat have a lower prevalence of *Campylobacter* contamination than chicken meat (<5% to 14%),[19-21] but a higher prevalence is found in animal offal such as liver,[22] thus making offal a valuable source of host-associated strains of *Campylobacter* in low-prevalence meats.

STUDY RATIONALE

In the United States, evidence from case-control studies have led to policy change, including changes to chicken slaughtering techniques. The incidence of human *Campylobacter* infection has declined in the US since this policy was introduced in 1997.[23] More recently, evidence from source attribution analyses in New Zealand has led to the development of poultry production policies and practices aimed at reducing the risk of *Campylobacter* transmission via poultry food products.[24] New Zealand has seen a 74% reduction in the number of campylobacteriosis cases attributed to poultry in the region, as well as a 54% reduction in cases overall.[25]

Source attribution modelling enables us to determine which foods and animals are the most likely sources of infection with each *Campylobacter* strain type, and the proportion of cases attributed to each source. This can be done with simple proportional similarity index (PSI) calculations, or by using more complex models.[24] Source attribution also allows for human campylobacteriosis cases to be grouped by potential source, increasing the specificity of risk factor analyses. These source-assigned analyses combine the epidemiological information gained through the traditional case-control study with source attribution modelling to provide greater explanatory power to investigate locally-relevant risk factors.

OBJECTIVES

This study aims to:

1. Identify dietary, environmental and behavioural risk factors for *Campylobacter* infection in Australia
2. Strengthen the epidemiological evidence for previously identified risk factors in Australia
3. Identify strain-specific risk factors for infection using Whole Genome Sequencing (WGS) data from case isolates

HYPOTHESES

We will test several hypotheses regarding specific risk factors for *Campylobacter* infection in Australia. The hypotheses are based on exposures which have previously been identified as risk factors for *Campylobacter* infection in Australia as well as internationally.

We hypothesise that:

1. Persons who consume undercooked meats, particularly chicken, are at increased risk of infection.
2. Persons who consume offal are at increased risk of infection.
3. Persons who own companion animals (especially puppies) are at increased risk of infection.
4. Poor food hygiene and handling practices in the home increase the risk of infection.
5. Most human infections will be attributed to consumption of chicken meat.
6. There will be a high level of genetic diversity amongst *Campylobacter* strains.

STUDY DESIGN

We will conduct a case-control study including genomic testing over a two-year period in three sentinel sites: the state of Queensland (QLD), the Australian Capital Territory (ACT), and Hunter New England (HNE) region of New South Wales (Figure 1). Sporadic cases of culture-positive *Campylobacter* infection will be identified either through state notifiable disease registers, from local pathology service databases or local notification databases. An isolate from each case will be paired with epidemiological data from the case interview. One control will be recruited for each case who participates in the study, with trained interviewers conducting telephone interviews with both cases and controls. Participants will be interviewed using a questionnaire that has been specifically designed to collect information on known potential risk factors. This questionnaire will include a selection of questions being validated in a separate study (Liana Varrone, Validation of questions designed for gastroenteritis investigation). For cases, the questions will cover the seven days prior to the onset of illness, while controls will be questioned on the seven days prior to interview. Meanwhile, *Campylobacter* isolates will also be collected from food and animal samples. All human and non-human isolates will undergo whole genome sequencing for comparison in source attribution modelling. Data for this study will be collected from 1st March 2017 to 1st March 2019.

Figure 1. Map of Australian states and territories, showing the Hunter New England region. (Adapted from figure 1 in Eastwood *et al.* 2010)[26]

Patient and public involvement

To develop the study, we engaged state and territory health departments, food safety agencies and industry to establish research questions and methods. The process involved a dedicated workshop, followed by teleconferences and an iterative process of drafting study documentation. We also established a reference panel, which includes representatives from senior levels of government and industry bodies. No patients or other members of the public were involved in the development of this study.

STUDY POPULATION

The three sentinel sites cover a population of approximately 6.1 million people. Based on notification and diagnostic pathology data, we expect approximately 8,650 *Campylobacter* cases to be notified across these sites during the study period.

DEFINITION AND SELECTION OF CASES

Case definition

We define a case as a person from any of the three participating sites with a recent history of acute diarrhoea and a culture-positive stool result for *Campylobacter*.

SAMPLE SIZE

We used risk factor prevalence data from a previous national *Campylobacter* case-control study in 2001/2002 to estimate sample size for this study.[27] For example, the prevalence of chicken consumption among controls in 2001/2002 was 80%. A sample size of approximately 1,040 subjects (520 cases; 520 controls) would enable the study to detect an association between chicken consumption and illness with an odds ratio of 1.6, at 80% power and $\alpha = 0.05$, as reported in the previous study. Sample size estimates for other potential risk factors are listed in Table 1.

Table 1. Sample size estimates for an unmatched case-control study

Risk factor	Prevalence of exposure among controls (%)	Prevalence of exposure among cases (%)	Odds ratio	No. of required study subjects
Beef	78	85	1.6	960
Pork	52	60	1.4	1130
Lamb	42	50	1.4	1120
Chicken	80	87	1.6	1040
Offal	2.0	5.0	2.6	1154
Puppies	2.1	5.4	2.7	1040

80% power and $\alpha = 0.05$

From these calculations, we estimate that a study of 1,200 subjects (600 cases; 600 controls) will adequately detect significant associations of these magnitudes for potential risk factors of interest.

Queensland and Hunter New England sites will each enrol at least 250 cases into the study, while ACT will enrol at least 100 cases. Based on the previous Australian case-control study,[27] we expect approximately 80% of selected notified cases to be eligible and participate in the study (Table 2).

Table 2. Sampling method for cases in each site

State	Expected number of notified cases during study period	Estimated cases from participating pathology laboratory	Culture +ve cases	Sequential sampling of notified cases	Total no. of cases	Expected no. to be recruited (~ 80% participation rate)
QLD	7000	2800 (40%)	1260 (45% in QLD)	Select every 4th case	315	250
ACT	600	130	130	Include all notified cases	130	100
NSW (Hunter New England)	~1050	313	313	Include all notified cases	313	250
Total	**8650**	**3243**	**1703**		**758**	**600**

In Queensland, we will obtain cases from one private pathology provider reporting approximately 40% of the state's *Campylobacter* notifications. We estimate that this provider will notify 2,800 cases during the study period with an estimated 45% of these being culture-positive (1,260 notified cases). In ACT, approximately 600 *Campylobacter* notifications are expected during the study period; 130 are expected from the participating pathology laboratory. In Hunter New England, approximately 1,050 *Campylobacter* notifications are expected during the study period; 313 of these notifications will be from the participating pathology laboratory.

Enrolment of Cases

We will enrol all cases who meet the eligibility criteria (Table 3). Each site will check for new notifications of culture-positive *Campylobacter* infection daily, with only culture-positive *Campylobacter* cases eligible for this study. If a case refuses to participate in the study, we will select a subsequent case for inclusion. Enrolment of cases will depend on consent from the patient, or in the event of a child aged less than 18 years, consent from either one of the parents or the child's guardian. We will interview cases as soon as possible by telephone, preferably within two weeks of notification from the laboratory. It will be at the parent's or guardian's discretion as to whether a

child aged between 15 and 17 years is interviewed directly. The parent or guardian will be interviewed for cases aged less than 15 years.

Table 3. Eligibility criteria for cases and controls

Criteria	Cases	Controls
Had diarrhoea (≥3 loose bowel movements in 24hrs)	Include	Exclude
Known date of illness onset	Include	N/A
Household members positive for *Campylobacter* in 4 weeks prior to onset of illness	Exclude	Exclude (4 weeks prior to interview date)
Household members experiencing diarrhoea in 4 weeks prior to onset of illness	Exclude	Exclude (4 weeks prior to interview date)
Travelled outside of Australia in 2 weeks prior to onset of illness	Exclude	Exclude (2 weeks prior to interview date)
Travelled interstate for the entire 2 weeks prior to onset of illness	Exclude	Exclude (2 weeks prior to interview date)
Can't speak English	Exclude	Exclude
Not able to answer questions for some other reason (e.g. intellectually disabled)	Exclude	Exclude
Not contactable after 6 telephone attempts	Exclude	Exclude
Live outside the catchment areas	Exclude	Exclude
Do not have a telephone number available for their primary residence, or a mobile phone	Exclude	Exclude
An enteric pathogen other than *Campylobacter* was isolated/detected in their stool (excluding *Blastocystis hominis* and *Dientamoeba fragilis*)	Exclude	N/A

DEFINITION AND SELECTION OF CONTROLS

We will recruit controls from notified cases of influenza, frequency matched by sex, age group and geographical area of residence by Statistical Area Level 4 (SA4). These controls will be selected with a delay of at least six months from their influenza infection to ensure that controls have returned to eating their customary diet.

Each participating site (QLD, ACT or HNE) will establish a database of controls (previous influenza cases). All cases of influenza notified to the health department in each site between 1st January and 31st December 2017 will be entered into this control database. The age bands are 0-4 years, 5-14 years, 15-34 years, 35-54 years, 55-74 years, and ≥75 years. An appropriate control will be randomly selected from the database within 30 days of interview of the notified case.

Case and control recruitment

Interviewers trained in computer-assisted telephone interviewing (CATI) will conduct telephone interviews. A maximum of six attempts will be made to contact any one case or control, with no more than three attempts in any one day. Three calls will be attempted between 9:00am and 3:59pm, and three attempts between 4:00pm and 8:00pm. A text message will be sent to the potential participant after three failed call attempts, indicating that Public Health is trying to contact them. This protocol will be continued until the person is enrolled or excluded.

QUESTIONNAIRES

We will use specific case and control questionnaires for all participants (see Appendix 1). Cases will be asked additional questions about the clinical course of their illness and treatment. Interviewers will ask identical questions regarding exposures such as foods consumed, dining locations, water sources, domestic food handling techniques and exposure to animals of cases and controls. Questions on foods consumed, dining locations, water consumed, animal and pet exposures will be asked based on a seven-day history. Questions on international travel will be asked based on a two-week history. Antibiotic and antacid consumption, immunosuppressive treatment and household history of diarrhoea will be based on a four-week history. Questions on food handling and general kitchen practices will be based on usual practices rather than recent history. Demographic information will be collected from cases and controls. Contact information required to conduct interviews will be stored in a password-protected Excel document with only those needing to contact individuals given access. Piloted questionnaires were modified to remove repetitions, improve clarity, and to ensure that interviews could be conducted within 20 minutes.

DATA HANDLING & RISK FACTOR ANALYSIS

We will undertake descriptive reporting of campylobacteriosis incidence by person, place and time. We will also describe the severity of symptoms, treatment, and burden of illness.

Risk factor analysis will involve the examination of two-by-two contingency tables with chi square or exact tests to determine the presence of univariable associations between variables and disease. To measure the strength of an association, we will estimate odds ratios and calculate 95% confidence

intervals in a univariable analysis, followed by multivariable logistic regression modelling to adjust for potential confounders. Risk factors selected for inclusion in the regression model will include age, season and geographic area, variables with a significant univariable association with disease, and variables with a P-value ≤0.25 that are biologically plausible and of interest to the research team.

LABORATORY ANALYSES

Human samples

As outlined in Table 2, it is expected that 250 human isolates from Hunter New England, 250 from Queensland, 100 from Victoria and 100 from ACT will be sequenced. The initial isolation and confirmation of *Campylobacter* infection will be performed locally in each State/Territory. Only samples with a pure and viable culture will undergo WGS.

Animal and food samples

We will collect samples from chicken meat (covering the two production methods of continually housed and free range/housed), beef, lamb, pork, and from pet dogs. Given low prevalence of *Campylobacter* in meats other than chicken, samples will be collected from offal (preferably liver) from bovine, ovine and porcine sources to ensure sufficient positive samples are obtained for the study. Given the rising importance of chicken liver pate as a source of outbreaks in Australia,[28] chicken offal will also be sampled. Sample sizes by source are based on data from two states to ensure 50 positive samples per food source, and 30 samples in companion animals (Table 4). We will also contact veterinary clinics and teaching hospitals to ensure sufficient *Campylobacter*-positive samples from dogs. Water samples have been omitted from the genomic aspect of this study due to logistical constraints in sampling untreated water sources across the large geographical area involved in this study, and the complexity of designing an appropriate sampling frame. As there is a lack of evidence implicating municipal drinking water as sources of *Campylobacter* infection in Australia[1 27] we excluded water sampling from this study.

Table 4. Sampling to ensure 50 isolates per food source and 30 isolates from companion animals

	Foods						Animals	
	Chicken			**Beef**	**Lamb**	**Pork**	**Dogs**	**Total**
	Continually housed	*Free-range*	*Offal*	*Offal*	*Offal*	*Offal*		
Assumed prevalence	0.7	0.7	0.7	0.14	0.6	0.22	0.2	
Samples required	72	72	72	286	100	272	150	**1041**
Positive isolates	50	50	50	40	60	60	30	**330**

The initial isolation and confirmation of *Campylobacter* will be performed locally at laboratories in each State/Territory, with isolates forwarded to the Microbiological Diagnostic Unit Public Health Laboratory for WGS, except Queensland isolates which will be sequenced at Queensland Health. To detect seasonal and temporal variation in *Campylobacter* genetic types, 1041 non-human samples (estimated to produce 330 *Campylobacter* isolates) will be collected over a period of one year in Queensland, and two years in New South Wales. To assess latitudinal variation in chicken meat samples across eastern Australia, 105 chicken samples (70 chicken meat and 35 chicken offal) will be collected over a six-month period in Victoria. Food samples will be collected monthly from retail premises, using protocols from surveys undertaken in 2014 by partner organisations, with a pilot of 30 isolates in Queensland.

We will also collect an additional 20-30 human isolates from four additional Australian jurisdictions not participating in this case-control study to undergo WGS. This will be done over a two-month period that overlaps with the case-control study sample collection, and is planned to help inform the generalisability of the case-control study.

SEQUENCING AND SEQUENCE DATA PROCESSING

Campylobacter isolates selected for sequencing will be repurified on solid medium and a single colony selected for preparation of genomic DNA. A sequencing library will be prepared from the genomic DNA for sequencing on the Illumina sequencing platform (MiSeq or NextSeq). A sample of the selected colony will be regrown and cryopreserved (resuspended in liquid medium supplemented with 10% Glycerol and stored at -80^{o}C). In some cases, *Campylobacter* enrichment cultures will be cryopreserved to enable future investigation of the genetic diversity of *Campylobacters* present. The short-read, paired end dataset produced by the Illumina Instrument from the genomic DNA of each isolate will be processed to produce a draft genome sequence for the isolate using a *de novo* assembler such as MEGAHIT.[29] The draft genome sequence will be annotated using Prokka.[30] We will use the draft genome sequence to perform the initial sub-species classification by deriving a multilocus sequence type (MLST) using the "*Campylobacter jejuni/coli*" typing scheme (pubmlst.org). Again, using the draft genome sequence, further typing e.g. virulence factors (http://www.mgc.ac.cn/VFs/) or antimicrobial resistance genotype (https://cge.cbs.dtu.dk/services/ResFinder/) will be performed using Abricate (https://github.com/tseemann/abricate). We will perform comparative genomics to examine the genetic relationships between selected subgroups of isolates in more detail using Nullarbor (https://github.com/tseemann/nullarbor).

SOURCE ATTRIBUTION MODELLING

We will analyse the epidemiological data within designated MLST groups or other typing groups derived from the genomic sequence data. Source attribution modelling and source-assigned analyses will be conducted.

Source attribution models combine typing data from isolates from food, animal and humans to estimate the proportion of human infections that can be attributed to animal and food reservoirs.[31] [32] Once inferred MLSTs have been ascertained, the proportional similarity index[25] will be used to assess similarities by source. We will then undertake source attribution analyses by adapting the asymmetric island model which has previously been applied to MLST data[25] [33] using Markov Chain Monte Carlo (MCMC) methods[34] implemented using the free software WinBUGS.[35] These methods will first be applied to MLST data extracted from whole genome sequences (the aforementioned "inferred MLSTs"), and then compared to structured phylogenetic modelling approaches[36] [37] that provide scope to infer inter-host transmission.

We will then group cases according to putative source based on these source attribution methods.[38] For example, all isolates attributed to chicken will be grouped together, regardless of differing strains. These cases attributed to chicken will then be compared to all controls in a risk factor analysis to produce a source-assigned analysis.

SPATIAL CLUSTERS AND TEMPORAL TRENDS

We will use newly-designated WGS-based MLSTs to assess heterogeneity in isolates from food sources and companion animals in Queensland and New South Wales, and in isolates from chicken meat and humans across Queensland, New South Wales, Victoria and ACT. A two-year sampling framework in New South Wales, one year of sampling in Queensland, and previous survey work in these states will allow us to assess the extent of seasonal and temporal trends. Postcode-level data associated with human illnesses will be used to detect space-time clusters using a scan statistic implemented in the free software SaTScan, at the Statistical Area 1 level.[39] We will use a retrospective space-time permutation model to detect high risk clusters by comparing the observed number of illnesses to the expected number in that geographic zone and time-period.[40]

STUDY LINKAGES AND COLLABORATIONS

The CampySource Project Team comprises three working groups and a reference panel. The working groups focus on: food and animal sampling, epidemiology and modelling, and genomics. The reference panel includes expert representatives from government and industry.

The study is supported by the following partner organisations: the Australian National University, Massey University, University of Melbourne, Queensland Health, Queensland Health Forensic and Scientific Services, New South Wales Health, Hunter New England Health, Victorian Department of Health and Human Services, Food Standards Australia New Zealand, Commonwealth Department of Health and AgriFutures Australia – Chicken Meat Program.

CampySource is also supported by collaboration with the following organisations: ACT Health, Sullivan Nicolaides Pathology, University of Queensland, Primary Industries and Regions South Australia, Department of Health and Human Services Tasmania, Meat and Livestock Australia, and New Zealand Ministry for Primary Industries.

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While undertaking studies, LV is supported through an Australian Government Research Training Program (RTP) Scholarship.

DECLARATIONS

Ethics approval and consent to participate

Informed Consent

A suitably trained interviewer will inform potential participants about the purpose, methods and demands of the study. We will obtain verbal consent from all study participants or their guardians.

Persons aged 18 years and older will be interviewed following informed consent. It will be at the parent's or guardian's discretion as to whether a child aged between 15 and 18 years is interviewed directly, following informed parental/guardian consent. Parents/guardians will be interviewed for cases aged less than 15 years, after informed consent is obtained.

Confidentiality

All information and identifiers will be kept confidential. Names and personal identifiers will be collected and entered into computer records but will be password protected. No personal identifiers will be included in any published materials relating to this study. All hard copy questionnaires containing patient identifiers will be stored in locked filing cabinets in a secure location to which only study investigators and interviewers will have access.

Risks and Benefits

Participants will be informed there are no individual benefits associated with the study and that participation is voluntary. Failure to participate or a withdrawal of participation will not affect any future treatment. There is also no risk to the patient, and the only cost is time spent – approximately 20 minutes – being interviewed. They may refuse to answer any of the questions or stop at any time.

Animal Ethics

All procedures involving live animals will be performed in accordance with a protocol approved by the University of Melbourne's Animal Ethics Committee (ethics ID: 1714156).

Consent for publication

Not applicable.

Availability of data and materials

The Illumina read sets produced as part of this study will be published at INSDC (Sequence Read Archive (DDJB/NCBI) or the European Nucleotide Archive (EMBL-EBI))

Competing interests

No authors have any competing interests to declare.

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Author contributions

MDK conceived the original idea for this study. All authors contributed to the study design and analysis plan. LV and RJS wrote the first draft with contributions from all authors. LV, RJS, LS, MDK and KG were involved in multiple revisions. The final version of the manuscript was approved by all authors.

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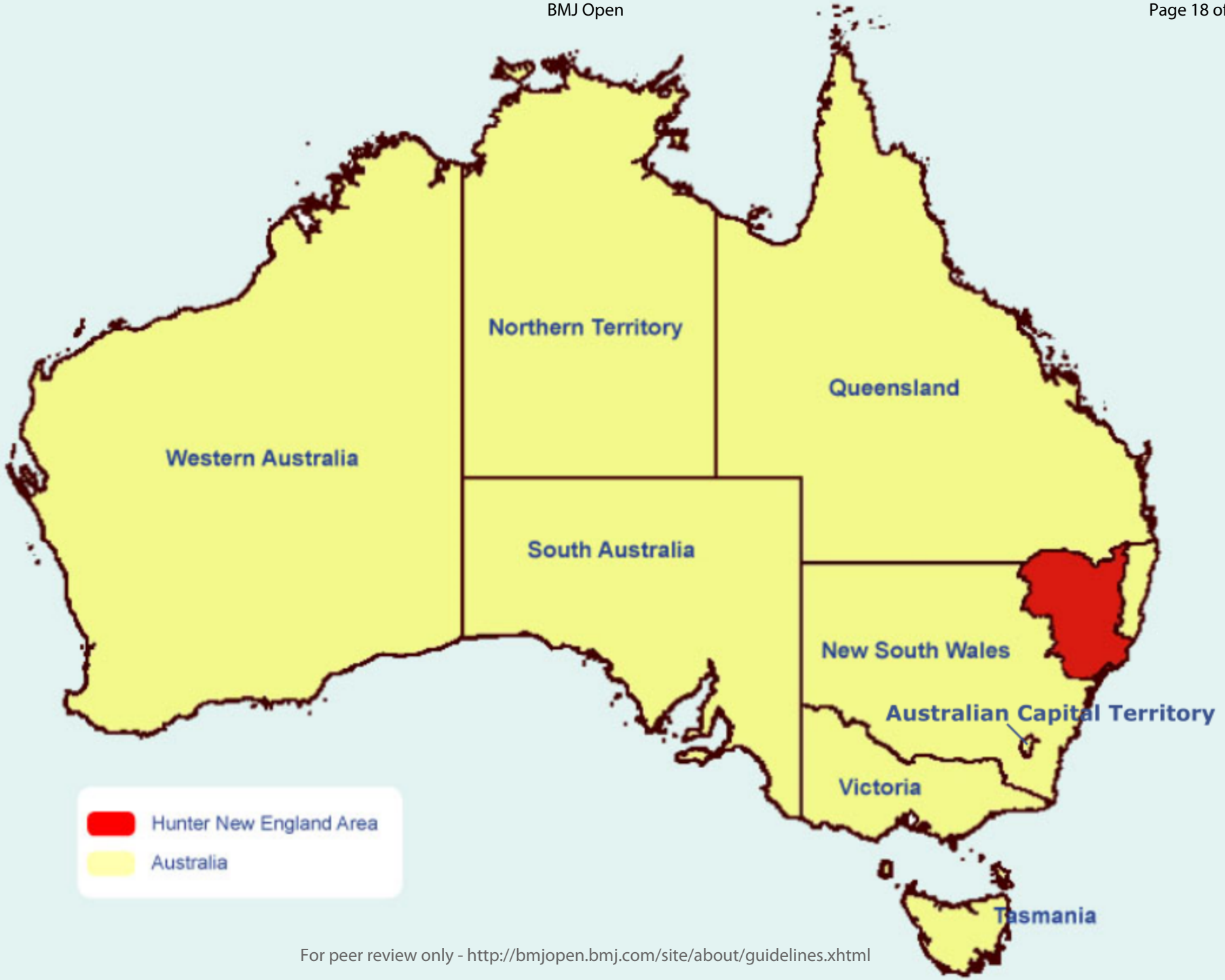

Refusal	☐
Ineligible	☐
ID Number	☐☐☐☐☐☐
Interview Date	☐☐☐☐☐☐
Interview Start Time	_____
Data entered	☐
Date	☐☐☐☐☐☐
Data checked	☐
Date	☐☐☐☐☐☐

Source Attribution of *Campylobacter* in Australia Study

Case Questionnaire

INTRODUCTION

Interviewer Note:	If case is less than 15 years of age you will need to speak to parent or guardian most familiar with the eating habits of the child. If case is aged between 15–17 years you will need to obtain parent or guardian consent prior to interview. Please note that for subjects under the age of 15 years, questions relate to the case, not the person being interviewed unless specified in the body of the questionnaire.

"Hello, my name is <Interviewers Name> and I am calling on behalf of [Queensland Health / ACT Health / Hunter New England Public Health Unit]."
"May I please speak with <name of case> or <name of case's mother/father>?"

Interviewer Note:	When the case comes to the phone then repeat the introduction and proceed with the explanatory statement. If the case is unavailable then arrange an alternative time for the interview

"The Australian National University in conjunction with the [state health department] is conducting a study of *Campylobacter* infection in Australia to identify possible causes. *Campylobacter* infections are notifiable to health departments in each state and territory throughout Australia. We understand that you recently experienced an illness due to the *Campylobacter* bacteria. We would like to find out more about your *Campylobacter* illness. Your participation is voluntary, all responses are confidential and if there are any questions you do not wish to answer, just say so."
"Would you be prepared to answer some questions about your illness and activities prior to your illness? The questions will take approximately 20 minutes." Yes ▪ No ▪

"In this study, we will collect information on foods you ate and activities you undertook prior to your illness. Your participation is voluntary and you can stop at any time. No individual information will be presented in any reports or presentations. Partners in this research will comply with the Australian Privacy Act 1988. An information sheet about this research project is available on the ANU website.

"Would you like me to send you a copy or provide you with the link?" Yes ▪ No ▪

If yes, "Could you give me an address/email?" _____

"This study has been approved by the Australian National University Ethics Committee (Protocol 2016/426). If you have any concerns please direct them to
Human Research Ethics Committee
Research Services Office, Chancellery 10B
The Australian National University, ACT 2601
Tel: 6125 7945 Fax: 6125 4807
Email: Human.Ethics.Officer@anu.edu.au"

"Do you have any further questions about the project?" Yes ▪ No ▪

Do you agree to participate in the project? Yes ▪ No ▪

"Do you have the time right now to answer these questions?"

If NO, arrange an alternative time to phone back to conduct the interview
If YES, continue

1. ELIGIBILITY QUESTIONS

"Because I will be asking about specific dates around the time of your illness, it may be helpful for you to have a calendar or diary in front of you. Do you need a few minutes to get these?"

Yes, I will get one no ☐
No, I already have one with me ☐
Don't have access to a calendar ☐

The first few questions we'll be asking you are about some symptoms that are associated with [your/their] illness.

1. For the purposes of this study, we define diarrhoea as 3 or more loose stools or bowel movements in any 24-hour period. When you had your *Campylobacter* infection, did you have diarrhoea?

Yes	1	**Go to Q2**
No	2	**Check ineligible box then END INTERVIEW**
Don't know/Not sure	7	**Check ineligible box then END INTERVIEW**

1a During this diarrhoeal illness, what was the maximum number of stools or bowel movements you had in any 24 hour period?

0-2	1	**Check ineligible box then END INTERVIEW**
3-5	2	*(If response = '0–2', then recode Q.1 as = '2')*
6-10	3	
11-20	4	
More than 20	5	
Don't know/Not sure	7	

2. For how many days did your diarrhoea last? ☐☐ **DAYS**

Don't know/Not sure ☐ 77

CALCULATE PRIOR TO INTERVIEW

Date stool specimen collected ☐☐ Day ☐☐ Month ☐☐ Year

3. Could you please let me know what the date was when your diarrhoea began?

☐☐ Day ☐☐ Month ☐☐ Year

(If person is unsure of date then prompt with date of stool specimen)

Don't know/Not sure ☐ 7 **Check ineligible box then END INTERVIEW**

I will now just enter a couple of other dates that we will be talking about throughout the interview. I won't be a moment....

Interviewer Note:	Refer to your calendar to determine the interval from DATE 4 WEEKS BEFORE DIARRHOEA BEGAN to DATE 1 DAY BEFORE DIARRHOEA BEGAN.

4. **In the 4 weeks before your illness began, that is from <DATE 4 WEEKS BEFORE DIARRHOEA> through <DATE 1 DAY BEFORE DIARRHOEA BEGAN>, did anyone else in your household test positive for *Campylobacter*?**

Yes ..... ☐ 1 **Check ineligible box then END INTERVIEW**
No ..... ☐ 2
Don't know/Not sure ..... ☐ 7

5. **In the 4 weeks before your illness began, that is from <DATE 4 WEEKS BEFORE DIARRHOEA> through <DATE 1 DAY BEFORE DIARRHOEA BEGAN>, did anyone else in your household have diarrhoea?**

Yes ..... ☐ 1 **Check ineligible box then END INTERVIEW**
No ..... ☐ 2
Don't know/Not sure ..... ☐ 7

Interviewer Note:	Refer to your calendar to determine the interval from DATE 2 WEEKS BEFORE DIARRHOEA BEGAN to DATE 1 DAY BEFORE DIARRHOEA BEGAN.

6. **In the 2 weeks before your illness began, that is from <DATE 2 WEEKS BEFORE DIARRHOEA> through <DATE 1 DAY BEFORE DIARRHOEA BEGAN>, did you travel overseas or interstate?**

INTERVIEWER NOTE:
IF participant answers "yes",
1. Clarify if the travel was overseas or interstate
2. If travel was interstate:
Clarify the length of time spent interstate in the time period just mentioned

Options to select:
A. If the participant has travelled overseas or spent the whole two weeks interstate: (Select option Yes)
B. If the participant has travelled interstate only for a portion of the time: (Select option No)

Yes..... ☐ 1 **Check ineligible box then END INTERVIEW**
No..... ☐ 2
Don't know/Not sure..... ☐ 7

2. HEALTH QUESTIONS

7. During this illness, did you have any of the following symptoms?

		Yes	No	DK/NS
a.	Fever	☐ 1	☐ 2	☐ 7
b.	Vomiting	☐ 1	☐ 2	☐ 7
c.	Stomach cramps	☐ 1	☐ 2	☐ 7
d.	Blood in your stool	☐ 1	☐ 2	☐ 7
e.	Nausea	☐ 1	☐ 2	☐ 7
f.	Headache	☐ 1	☐ 2	☐ 7
g.	Muscle/body aches	☐ 1	☐ 2	☐ 7

8. Did you take any antibiotics as a result of this illness?

Yes	☐ 1	
No	☐ 2	**Go to Q. 10**
Don't know/Not sure	☐ 7	**Go to Q. 10**

9. What antibiotic(s) were you taking? [Ask person to get tablet bottle, if possible]

Azithromycin	☐
Ciprofloxacin	☐
Norfloxacin	☐
Erythromycin	☐
Doxycycline (also known as Doxy or Vibramycin	☐
Other (please specify)	☐ Specify(_____)
Don't know/Not sure	☐

10. Were you admitted to hospital overnight because of this illness?

Yes	☐ 1	
No	☐ 2	**Go to Q. 12**
Don't know/Not sure	☐ 7	**Go to Q. 12**

11. If yes, **for how many nights were you hospitalised?** ☐ NIGHTS

Don't know/Not sure ☐ 7

Interviewer Note:	Refer to your calendar to determine the interval from DATE 4 WEEKS BEFORE DIARRHOEA BEGAN to DATE 1 DAY BEFORE DIARRHOEA BEGAN.

"For the next few questions, I would like to ask you about events which may have occurred in the 4 weeks before your illness began, so again that's from <DATE 4 WEEKS BEFORE DIARRHOEA BEGAN> to <DATE 1 DAY BEFORE DIARRHOEA BEGAN>."

12. In those 4 weeks, were you taking any antibiotics?

Yes	☐ 1	
No	☐ 2	**Go to Q. 14**
Don't know/Not sure	☐ 7	**Go to Q. 14**

Interviewer Note:	If person can't remember the name of the antibiotic(s), check the DK/NS box and leave the space blank.

13. What antibiotic(s) were you taking? [Ask person to get tablet bottle, if possible]

		DK/NS	**What date did you stop taking these?**
a.	Antibiotic 1_____	☐ 7	☐☐ ☐☐ (DD/MM) ☐ 7 **DK/NS**
b.	Antibiotic 2_____	☐ 7	☐☐ ☐☐ (DD/MM) ☐ 7 **DK/NS**
c.	Antibiotic 3_____	☐ 7	☐☐ ☐☐ (DD/MM) ☐ 7 **DK/NS**
d.	Antibiotic 4_____	☐ 7	☐☐ ☐☐ (DD/MM) ☐ 7 **DK/NS**

14. In those 4 weeks, were you taking any regular medication that decreases stomach acid?

Yes.....	☐ 1	
No.....	☐ 2	**Go to Q. 16**
Don't know/Not sure.....	☐ 7	**Go to Q. 16**

15. Did you take any of the following in the 4 weeks prior to illness?

Histamine-2 (H_2) Receptor blocker

		Yes	**No**	**DK/NS**
a.	Zantac (Ranitidine)……	☐ 1	☐ 2	☐ 7
b.	Tagamet (Cimetidine)……	☐ 1	☐ 2	☐ 7
c.	Pepcid (Famotidine)……	☐ 1	☐ 2	☐ 7
d.	Axid (Nizatidine)……	☐ 1	☐ 2	☐ 7

Proton Pump Inhibitor

a.	Losec (Omeprazole)……	☐ 1	☐ 2	☐ 7
b.	Nexium (Esomeprazole)……	☐ 1	☐ 2	☐ 7
c.	Somac (Pantoprazole)……	☐ 1	☐ 2	☐ 7
d.	Pariet (Rabeprazole)……	☐ 1	☐ 2	☐ 7
e.	Zoton (Lansoprazole)……	☐ 1	☐ 2	☐ 7

16. Have you ever been told by a doctor that you have any other long lasting condition or chronic illness in which diarrhoea or vomiting is a major symptom? (e.g. Crohn's disease, irritable bowel syndrome, ulcerative colitis, or stomach or oesophagus problems)

Yes.....	☐ 1	Specify(_____)
No.....	☐ 2	
Don't know/Not sure.....	☐ 7	

17. In the 4 weeks before onset of illness, did you take or receive any of the following?

INTERVIEWER NOTE:
Cyclosporine ("it's an immunosuppressant")

		Yes	No	DK/NS
a.	Prednisone or other steroids not used on your skin……..…	☐ 1	☐ 2	☐ 7
b.	Cyclosporine ……..……..…..	☐ 1	☐ 2	☐ 7
c.	Chemotherapy……..……..	☐ 1	☐ 2	☐ 7
d.	Radiation therapy……	☐ 1	☐ 2	☐ 7

3. EXPOSURES

Interviewer Note: Refer to your calendar to determine the interval from the DATE 7 DAYS BEFORE DIARRHOEA BEGAN to the DATE 1 DAY BEFORE DIARRHOEA BEGAN

A. WATER

"I'm now going to ask you some questions about water that you consumed in the 7 days before your diarrhoea began, that is from [diarr_7_days_prior] to [diarr_1_day_prior].

18. What is your main source of drinking water at home? (select one only)

INTERVIEWER NOTE:
Only read out options if they're unsure

a.	A rainwater tank……	☐ 1	
b.	A river or stream ……	☐ 1	
c.	A private well, bore hole, or spearpoint……	☐ 1	
d.	A carrier or tank truck……	☐ 1	
e.	Municipal water supply (tap water)…….	☐ 1	
f.	Purchased bottle water……	☐ 1	
g.	Other water supply……	☐ 1	Specify (_____)
h.	Don't know/Unsure……	☐ 1	

Interviewer Note: If person answered "Yes" to "Municipal water supply" or "Purchased bottle water", **skip to Q.21**

19. Do you usually treat your main source of drinking water before drinking?

If Required PROMPT: Some examples are chlorination, filtration, boiling and UV treatment of the water

Yes……	☐ 1	Specify (_____)
No……	☐ 2	**Go to Q. 21**
Don't know/Not sure……	☐ 7	**Go to Q. 21**

20. Which of the following treatments are in place? (select all that apply)

		Yes	**No**	**DK/NS**
a.	Chlorination……	☐ 1	☐ 2	☐ 7
b.	Filtration……	☐ 1	☐ 2	☐ 7
c.	Boiling……	☐ 1	☐ 2	☐ 7
d.	UV treatment……	☐ 1	☐ 2	☐ 7
e.	Other…… Specify (_____)	☐ 1	☐ 2	☐ 7
f.	Don't know……	☐ 1	☐ 2	☐ 7

21. Did you <u>drink</u> water from any of the following sources in the 7 days before onset of diarrhoea…?
(Select all that apply)

		Yes		No		DK/NS	
a.	A rainwater tank……..……	☐	1	☐	2	☐	7
b.	A river or stream ……	☐	1	☐	2	☐	7
c.	A private well, bore hole, or spearpoint……	☐	1	☐	2	☐	7
d.	A carrier or tank truck……	☐	1	☐	2	☐	7
e.	Municipal water supply (tap water)…….	☐	1	☐	2	☐	7
f.	Purchased bottle water……..	☐	1	☐	2	☐	7
g.	Other water supply…….	☐	1	☐	2	☐	7

Specify (_____)

B. DINING LOCATIONS

"The next few questions ask about places where you may have eaten food in the 7 days before your illness began. So that is from [diarr_7_days_prior] to through [diarr_1_day_prior]"

22. During this time, did you eat any food prepared outside your home, for example takeaway, restaurant, someone else's home?

Yes	☐ 1	
No	☐ 2	**Go to Q 24**
Don't know/Not sure	☐ 7	**Go to Q 24**

23. Did you eat any food from the following places?

		Yes	No	DK/NS
a.	Café or restaurant	☐ 1	☐ 2	☐ 7
b.	Home cooked meal at someone else's home	☐ 1	☐ 2	☐ 7
c.	Kebab shop	☐ 1	☐ 2	☐ 7
d.	Other fast food/take away outlet	☐ 1	☐ 2	☐ 7

23a How many meals prepared outside of your home, were eaten during this 7 day period?

1-2 meals	☐ 1
3-4 meals	☐ 2
≥ 5 meals	☐ 3
Don't know/Unsure	☐ 7

C. DAIRY PRODUCTS

"I would now like to ask you about the dairy products you may have eaten in the 7 days before your diarrhoea began."

24. Did you drink any raw/unpasteurised milk or eat any products made from raw/unpasteurised milk?

INTERVIEWER NOTE:
Cold-pressed milk is pasteurised and is not to be included as "raw/unpasteurised".

		Yes	No	DK/NS
a.	Unpasteurised milk……	☐ 1	☐ 2	☐ 7
b.	Other products……	☐ 1	☐ 2	☐ 7
	Specify (_____)			

D. MEAT AND POULTRY

"I will now ask you some questions about meat and poultry that you may have eaten in the 7 days before your diarrhoea began, that is from <DATE 7 DAYS BEFORE DIARRHOEA BEGAN> through <DATE 1 DAY BEFORE DIARRHOEA BEGAN>."

25. During these 7 days, did you eat any of the following deli meats or cold cuts?

	Yes	No	DK/NS
a. Salami/mettwurst ……	☐ 1	☐ 2	☐ 7
b. Cabanossi/cabana/twiggy sticks……	☐ 1	☐ 2	☐ 7
c. Ham/chicken/turkey/beef……..……	☐ 1	☐ 2	☐ 7
d. Devon/frankfurts/cheerios..……	☐ 1	☐ 2	☐ 7
e. Liverwurst…….……..	☐ 1	☐ 2	☐ 7
f. Other …….. Specify (_____)	☐ 1	☐ 2	☐ 7

26. During these 7 days, did you eat any pate?

Yes..... ☐ 1
No..... ☐ 2 **Go to Q.29**
Don't know/Not sure..... ☐ 7

27. Was the pate eaten,

Chicken pate..... ☐ 1
Duck pate..... ☐ 2
Pork pate..... ☐ 3
Another type of pate..... ☐ 4 Specify (_____)
Don't know/Not sure..... ☐ 7

28. Was this pate homemade or purchased from a store?

Homemade..... ☐ 1
Store..... ☐ 2
Don't know/Not sure…..... ☐ 7

29. During these 7 days, did you eat any other meat or poultry? Like beef, lamb, chicken etc.

INTERVIEWER NOTE:

This does not include eggs

Yes..... ☐ 1
No..... ☐ 2 **Go to Q.49**
Don't know/Not sure..... ☐ 7

BEEF / VEAL

30. During these 7 days, did you eat any beef or veal?

Yes..... ☐ 1
No..... ☐ 2 **Go to Q. 32**
Don't know/Not sure..... ☐ 7

31. During the 7 days prior, did you eat any of the following beef or veal?

		Yes	No	DK/N	
a.	Minced beef dishes…… *(eg. bolognese sauce, pie, pastie, lasagne, hamburger patties, sausages)*	☐ 1	☐ 2	☐ 7	
b.	Kebabs/souvlaki……	☐ 1	☐ 2	☐ 7	
c.	Offal…… *(eg. tripe, liver, tongue)*	☐ 1	☐ 2	☐ 7	Specify (_____)
d.	Other…… *(eg. casserole, stir fry, steak, fillet, roast, beef strips)*	☐ 1	☐ 2	☐ 7	

PORK

32. During these 7 days, did you eat any pork?

Yes..... ☐ 1 Specify (_____)
No..... ☐ 2 **Go to Q. 34**
Don't know/Not sure..... ☐ 7

33. During the 7 days prior, did you eat any of the following pork?

		Yes	No	DK/N	
a.	Minced pork dishes…… *(eg. bolognese sauce, pie, pastie, lasagne, hamburger patties, sausages)*	☐ 1	☐ 2	☐ 7	
b.	Kebabs/souvlaki……	☐ 1	☐ 2	☐ 7	
c.	Offal…… *(eg. tripe, liver, tongue)*	☐ 1	☐ 2	☐ 7	Specify (_____)
d.	Other…… *(eg. casserole, stir fry, steak, fillet, roast, pork strips)*	☐ 1	☐ 2	☐ 7	

LAMB

34. During these 7 days, did you eat any lamb/mutton?

Yes..... ☐ 1 Specify (_____)
No..... ☐ 2 **Go to Q. 36**
Don't know/Not sure..... ☐ 7

35. During the 7 days prior, did you eat any of the following lamb/mutton?

		Yes	No	DK/N	
a.	Minced lamb/mutton dishes…… *(eg. bolognese sauce, pie, pastie, lasagne, hamburger patties, sausages)*	☐ 1	☐ 2	☐ 7	
b.	Kebabs/souvlaki……	☐ 1	☐ 2	☐ 7	
c.	Offal…… *(eg. tripe, liver, tongue)*	☐ 1	☐ 2	☐ 7	Specify (_____)
d.	Other…… *(eg. casserole, stir fry, steak, fillet, roast, lamb strips)*	☐ 1	☐ 2	☐ 7	

GAME MEAT

36. During these 7 days, did you eat any game meat like kangaroo, wallaby, venison or similar?

Yes…… ☐ 1 Specify (_____)
No…… ☐ 2
Don't know/Not sure…… ☐ 7

POULTRY

37. How often do you <u>usually</u> consume chicken/poultry meat?

3 or more days per week…… ☐ 1
1-2 days per week…… ☐ 2
Once per fortnight…… ☐ 3
Less often than once per fortnight…… ☐ 4
Never…… ☐ 5 **Go to Q.46**
Don't know/Not sure…… ☐ 7

38. During the 7 days before your illness began, did you eat any chicken or other poultry?

Yes…… ☐ 1
No…… ☐ 2 **Go to Q.46**
Don't know/Not sure…… ☐ 7

39. How many meals did you eat that contained chicken or other poultry in the 7 days prior to onset of diarrhoea?

1-2 meals…… ☐ 1
3-4 meals…… ☐ 2
≥ 5 meals…… ☐ 3
Don't know/Not sure…… ☐ 7

40. Did you consume any chicken or poultry at home?

Yes..... ☐ 1
No..... ☐ 2 **Go to Q.43**
Don't know/Not sure..... ☐ 7

41. Was the chicken or poultry purchased…?
(Select all that apply)

Raw and fresh..... ☐ 1
Raw and frozen..... ☐ 2
Pre-cooked..... ☐ 3
Don't know/Not sure..... ☐ 4

42. How was it stored before consumption…?
(Select all that apply)

INTERVIEWER NOTE:
(On the bench)
This is only to be used if they STORE their meat on the bench, this does not include defrosting their meat on the bench.

In the freezer..... ☐ 1
In the fridge..... ☐ 2
On the bench..... ☐ 3
Don't know/Not sure..... ☐ 4

43. Prior to cooking, was the chicken rinsed or washed under running water ….?

Yes..... ☐ 1
No..... ☐ 2
Don't know/Not sure..... ☐ 7

44. During this did time you eat any of the following cooked meats…..…?

	Yes	No	DK/NS
a. Chicken mince..…… *(including hamburger patties, sausages)*	☐ 1	☐ 2	☐ 7
b. Chicken kebabs……..	☐ 1	☐ 2	☐ 7
c. Chicken pieces with bones…. *(i.e. wings, drumsticks, whole chicken)*	☐ 1	☐ 2	☐ 7
d. Chicken pieces without bones. *(i.e. breast, tenderloins)*	☐ 1	☐ 2	☐ 7
e. Offal..……	☐ 1	☐ 2	☐ 7
specify: liver	☐ 1	other _____	
f. Duck……	☐ 1	☐ 2	☐ 7
g. Turkey……..…	☐ 1	☐ 2	☐ 7

45. During this time, on how many days did you eat poultry?

INTERVIEWER NOTE:
1. A pate is included
2. Eggs are excluded

Days:_____

46. During this time, on how many days did you eat meat (including poultry)?

INTERVIEWER NOTE:
Pate is included

Days:_____

47. During the 7 days prior to illness, did [you/they] eat any meat product, which was raw, rare or appeared undercooked?

Yes	☐ 1	
No	☐ 2	**Go to Q.49**
Don't know/Not sure	☐ 7	

48. Which of the following meats did [you/they] eat that was undercooked?

		Yes	No	DK/NS
a.	Chicken/poultry	☐ 1	☐ 2	☐ 7
b.	Beef or veal	☐ 1	☐ 2	☐ 7
c.	Pork	☐ 1	☐ 2	☐ 7
d.	Lamb/mutton	☐ 1	☐ 2	☐ 7
e.	Game meat	☐ 1	☐ 2	☐ 7
f.	Minced meat items *(eg. including sausages, hamburger patties)*	☐ 1	☐ 2	☐ 7
g.	Offal (specify type)_____	☐ 1	☐ 2	☐ 7
h.	Other meat Specify (_____)	☐ 1	☐ 2	☐ 7

49. How do you *prefer* the following meat to be cooked?

INTERVIEWER NOTE:
Raw: Not cooked at all
Rare: Mostly red
Medium: Pink through out
Well done: Brown through out

INTERVIEWER NOTE:
If participant answers Medium/Rare
select the rarer option.. e.g Rare

		Raw	Rare	Medium	Well done
a.	Chicken/Poultry……	☐ 1	☐ 2	☐ 3	☐ 4
b.	Beef/Veal……..	☐ 1	☐ 2	☐ 3	☐ 4
c.	Pork……	☐ 1	☐ 2	☐ 3	☐ 4
d.	Lamb……..……	☐ 1	☐ 2	☐ 3	☐ 4
e.	Hamburgers……	☐ 1	☐ 2	☐ 3	☐ 4
f.	Minced meat……	☐ 1	☐ 2	☐ 3	☐ 4

E. GENERAL KITCHEN PRACTICES

"I will now ask you several questions about the way food is usually prepared in your home. Remember, your participation is voluntary and you do not have to answer any of the questions if you don't want to."

50. How many times per week do you cook for members of your household?

INTERVIEWER NOTE:
This section around food prepared in the home refers to the person answering the survey (not necessarily the case or control)

0	1	**Go to Q.63**
1-5	2	
>5	3	
Don't know/Not sure	7	

51. Did you handle or prepare any raw meats in the kitchen in the 7 days before your diarrhoea began?

INTERVIEWER NOTE:
Refers to the person answering the survey

Yes	1	
No	2	**Go to Q.57**
Don't know/Not sure	7	

52. Did you handle or prepare raw chicken meat or chicken offal in the 7 days before your diarrhoea began?

INTERVIEWER NOTE:
Refers to the person answering the survey

Yes	1	
No	2	
Don't know/Not sure	7	

Interviewer Note: If person answered "No" to **both** Q.51 and Q.52 then **skip to Q.57**

53. After a knife is used to cut raw meat or poultry, which of the following options do you usually do?

INTERVIEWER NOTE:
Refers to the person answering the survey

	Continue using the knife as is	1	
	Rinse the knife before continuing to cook	2	
	Wipe the knife before continuing to cook	3	
	Wash the knife with detergent before continuing	4	
	Change to another knife	5	
	Other	6	Specify (_____)
	No one prepares meat	7	**Go to Q. 57**
Don't read	Don't know/Not sure	8	

54. After a cutting board is used to cut raw meat or poultry, which of the following options do you usually do?

INTERVIEWER NOTE:
1. Does not matter if water is hot or cold
2. Refers to the person answering the survey

Continue using the cutting board as is…… ☐ 1
Rinse the cutting board before continuing to cook……..... ☐ 2
Wipe the cutting board before continuing to cook……..... ☐ 3
Wash the cutting board with detergent before continuing……... ☐ 4
Change to another cutting board……..…. ☐ 5
Other…… ☐ 6

Specify (_____)

Don't read Don't know/Not sure.....…… ☐ 7

55. After handling raw meat or poultry in the kitchen, which of the following would you usually do before continuing to cook?

INTERVIEWER NOTE:
Refers to the person answering the survey

Wipe hands..…… ☐ 1
Quickly rinse hands under a running tap..…….. ☐ 2
Wash hands with soap and water…… ☐ 3
Other…… ☐ 4

Specify (_____)

Don't do anything about hands…… ☐ 6 **Go to Q.57**
Don't read Don't know/not sure.....…… ☐ 7

56. After washing hands during food preparation, what would you usually dry your hands on?

INTERVIEWER NOTE:
Refers to the person answering the survey

Paper towel …… ☐ 10
Sponge/cloth……..…… ☐ 11
Tea-towel /hand towel …….. ☐ 12
Apron…..…… ☐ 13
Don't dry hands…….…… ☐ 14
Other…… ☐ 15

Specify (_____)

Don't read Don't know/Not sure.....…….. ☐ 77

57. In the past 3 months, has anyone in the household cook meat on a BBQ?

Yes.....…….. ☐ 1
No.....…… ☐ 2 **Go to Q. 59**
Don't know/Not sure…….. ☐ 7 **Go to Q. 59**

58. After cooking on the BBQ, where would the cooked meat most likely be placed?

	Back on the same container……	☐ 1	
	Back on the same container after it has been rinsed with water ……..….	☐ 2	
	Back on the same container after it has been wiped off with a towel…..……	☐ 3	
	Back on the same container, after the container has been washed with soap and water…..…….	☐ 4	
	On a different container ……..……..	☐ 5	
	Other……...	☐ 6	Specify (_____)
Don't read	Don't know/not sure……	☐ 7	

F. ANIMAL AND PET EXPOSURE

"The next few questions are about contact with animals in the 7 days before your diarrhoea began."

59. During this time, did you keep or care for any of the following animals as pets?

INTERVIEWER NOTE:
Not to include one off contact

		Yes	No	DK/NS	Is any pet less than 6 months old? Yes	No	DK/NS
a.	Cat……..	☐ 1	☐ 2	☐ 7	☐ 1	☐ 2	☐ 7
b.	Dog……..	☐ 1	☐ 2	☐ 7	☐ 1	☐ 2	☐ 7
c.	Chickens……..	☐ 1	☐ 2	☐ 7	☐ 1	☐ 2	☐ 7
d.	Other birds……..	☐ 1	☐ 2	☐ 7	☐ 1	☐ 2	☐ 7
e.	Other ……..	☐ 1	☐ 2	☐ 7	Specify (_____)		
f.	Do not keep any pets……	☐ 7 **Go to Q.67**					

Interviewer Note: If person answered No/Don't know to Cat then skip to question 62

60. Do you feed your cat raw meat or bones?

Yes……	☐ 1	Specify (_____)
No……	☐ 2	**Go to Q. 62** (eg. chicken, beef, kangaroo, lamb etc.)
Don't know/Not sure……	☐ 7	**Go to Q. 62**

61. How often does your cat get fed raw meat or bones?

Daily……	☐ 1
Weekly……	☐ 2
Monthly……	☐ 3
Less often……	☐ 4
Don't know/Unsure……	☐ 7

Interviewer Note: If person answered No/Don't know to Dog then skip to question 65

62. Do you feed your dog raw meat?

Yes……	☐ 1	Specify (_____)
No……	☐ 2	(eg. chicken, beef, kangaroo, lamb etc.)
Don't know/Not sure……	☐ 7	

63. Do you feed your dog raw bones?

Yes……	☐ 1	Specify (_____)
No……	☐ 2	
Don't know/Not sure……	☐ 7	

Interviewer Note: If person answered No/Don't know to questions 62-63 then skip to question 65

64. How often does your dog get fed raw meat or bones?

Daily..... ☐ 1
Weekly..... ☐ 2
Monthly..... ☐ 3
Less often..... ☐ 4
Don't know/Not sure..... ☐ 7

65. Did you get any of your pets in the 4 weeks before your diarrhoea began?

Yes..... ☐ 1 Pet(s) (_____)
No..... ☐ 2
Don't know/Not sure..... ☐ 7

66. Were any of your own pets ill with diarrhoea in the 7 days before your diarrhoea began?

Yes..... ☐ 1 Pet(s) (_____)
No..... ☐ 2
Don't know/Not sure..... ☐ 7

67. In the 7 days before your diarrhoea began, did you have contact with household pet faeces or manure (eg. changing litter boxes or picking up pet faeces with a plastic bag)?

Yes..... ☐ 1 Pet(s) (_____)
No..... ☐ 2
Don't know/Not sure..... ☐ 7

68. Do you live on a farm/hobby farm including a property on acreage 5 acres or over?

Yes..... ☐ 1
No..... ☐ 2
Don't know/Not sure..... ☐ 7

69. In the 7 days before your diarrhoea began, did you visit a farm or petting zoo?

Yes..... ☐ 1 Specify (_____)
No..... ☐ 2 (eg. private farm, commercial farm, petting zoo etc.)
Don't know/Not sure..... ☐ 7

4. DEMOGRAPHICS

"I would now like to ask you a few final questions. Remember, your participation is voluntary and you do not have to answer any of the questions if you don't want to."

70. Is any language other than English spoken in your household?

	Yes	☐ 1	Specify (_____)
	No	☐ 2	
Don't read	Don't know/Not sure	☐ 7	
Don't read	Refused	☐ 9	

71. Are you of Aboriginal or Torres Strait Islander origin?

No	☐	1
Aboriginal	☐	2
Torres Strait Islander	☐	3
Both	☐	4
Don't read Don't know/Not sure	☐	7
Don't read Refused	☐	9

72. Which of the following places best describe where you live?

Inner city or urban area	☐	1
Suburban area	☐	2
Town	☐	3
Rural or remote area community	☐	4
Rural or remote area farm or property	☐	5
Don't read Don't know/Not sure	☐	7
Don't read Refused	☐	9

Interviewer Note: See definitions below.

Inner city area:……*housing close to the centre of a major/capital city*
Suburban area:……*housing area further from the centre of the city, which is characterised by the region being primarily a self-contained residential district.*
Town:……*community over 2000 people*
Rural or remote area community:……*community under 2000*
Rural or remote area farm or property

73. Does your occupation involve any of the following?

Working with raw meat *(eg. restaurants, butchery, abattoir etc.)*	☐	11
Working with animals *(eg. farmer, zookeeper, vet/nurse etc.)*	☐	12
Other type of occupation	☐	13
Retired	☐	14
CASE not of working age	☐	15
Don't know/Unsure	☐	17

74. What is the highest level of education reached by <u>anyone</u> in your household?

	Schooling to year 10 or below…..……..	☐ 12
	Secondary school, above year 10…….	☐ 13
	Technical or further educational institution…….	☐ 14
	(eg. TAFE, apprenticeship, college etc.)	
	University degree—Undergraduate ……	☐ 15
	University degree—Postgraduate (Masters, doctorate).	☐ 16
Don't read	Don't know/not sure.....	☐ 7
Don't read	Refused…….....….	☐ 9

"Now I am going to read you a list of income categories. Please stop me when a category best describes your total household income, before taxes, in the last financial year? That is the total figure for all household members."

75. Last year the total income for your household was….?

	Less than $25,000.....……	☐ 1
	$25,000 to $50,000.....……...	☐ 2
	Between $50,000 and $100,000……..	☐ 3
	Between $100,000 and $150,000……..	☐ 4
	More than $150,000.....……...	☐ 5
Don't read	Don't know/Not sure.....……..	☐ 7
Don't read	Refused.....…..……..	☐ 9

76. As part of this research we are planning to do a follow-up study. Would you be happy for us to contact you in ~6 months' time?"

Yes.....……...	☐ 1	
No.....……...	☐ 2	**Skip to end of questionnaire**

Interviewer Note:	If person answered No to Q 76 then skip to the end of the questionnaire

Details required:

Name:_____

Phone number:_____

Email address:_____

"That's my last question. Thank you very much for your time and cooperation."

COMPLETE AFTER INTERVIEW

Interviewer initials _____

Interview stop time _____

Length of interview _____MINUTES

Respondent recall:

Poor	☐ 1
Fair	☐ 2
Average	☐ 3
Good	☐ 4
Excellent	☐ 5

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Investigating locally-relevant risk factors for Campylobacter infection in Australia: protocol for a case-control study and genomic analysis

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Investigating locally-relevant risk factors for *Campylobacter* infection in Australia: protocol for a case-control study and genomic analysis

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ABSTRACT

Introduction:

The CampySource project aims to identify risk factors for human *Campylobacter* infection in Australia. We will investigate locally-relevant risk factors and those significant in international studies in a case-control study. Case isolates and contemporaneous isolates from food and animal sources will be sequenced to conduct source attribution modelling, and findings will be combined with the case-control study in a source-assigned analysis.

Methods and analysis:

The case-control study will include 1,200 participants (600 cases and 600 controls) across three regions in Australia. Cases will be recruited from campylobacteriosis notifications to health departments. Only those with a pure and viable *Campylobacter* isolate will be eligible for selection to allow for whole genome sequencing of isolates. Controls will be recruited from notified cases of influenza, frequency matched by sex, age group and geographical area of residence. All participants will be interviewed by trained telephone interviewers using a piloted questionnaire.

We will collect *Campylobacter* isolates from retail meats and companion animals (specifically dogs), and all food, animal and human isolates will undergo whole genome sequencing. We will use sequence data to estimate the proportion of human infections that can be attributed to animal and food reservoirs (source attribution modelling), and to identify spatial clusters and temporal trends. Source-assigned analysis of the case-control study data will also be conducted where cases are grouped according to attributed sources.

Ethics and dissemination:

Human and animal ethics have been approved. Genomic data will be published in online archives accompanied by basic metadata. We anticipate several publications to come from this study.

KEYWORDS

Campylobacter, case-control study, risk factors, Australia, whole genome sequencing, source attribution, source-assigned analysis

ARTICLE SUMMARY

Strengths and limitations of this study

- Case-control study is well-powered to identify locally-relevant risk factors.
- Linking genomic data to the case-control study strengthens the analysis by enabling source attribution and source-assigned analyses to be conducted.
- Case-control questionnaire questions are being validated in a separate study, demonstrating the reliability of participant recall.
- Potential reporting bias due to inaccurate recall of study participants.
- Case-control study lacks efficiency for risk factors with high levels of exposure in the study population.

INTRODUCTION

Campylobacter infection is the most commonly notified cause of foodborne gastroenteritis in Australia,[1-3] as well as a leading cause of bacterial gastroenteritis world-wide.[4] At the introduction of Australia's National Notifiable Diseases Surveillance System (NNDSS) in 1991 the incidence rate of notified campylobacteriosis cases was 79.1/100,000 population,[5] and despite notification rates plateauing in recent years, incidence had risen to 139.7/100,000 population in Australia in 2015,[5] with an estimated 10 cases for every notified case within the community.[6] By comparison, the incidence rate of campylobacteriosis in New Zealand in 2014 was 150.3/100,000 population,[7] with an estimated 10-30 cases in the community for every notified case.[8] *Campylobacter* notification rates in Australia and New Zealand are still among the highest in the world across high-income countries. Most countries in the European Union consistently report annual campylobacteriosis notification rates below 100/100,000 population.[2]

Two species of *Campylobacter—Campylobacter jejuni* and *C. coli*—contribute to approximately 95% of human campylobacteriosis.[9] These *Campylobacter* species are commonly detected in sewage and surface water,[10] reside in the gastrointestinal tract of birds and animals,[11] and are frequently found in raw meat, particularly poultry, and raw milk.[12 13] Campylobacteriosis is mostly foodborne, with an estimated 77% of cases transmitted via food consumption in Australia.[14 15] Direct and indirect zoonotic transmission can occur via animal contact (direct) or faecally-contaminated water or environments (indirect). Person-to-person transmission is considered rare.[16] The majority of cases are thought to be sporadic, with outbreaks less commonly detected.[17] Most outbreaks are linked to the consumption of poultry, raw milk, or contaminated water.[17 18]

Targeted control of foodborne bacterial pathogens generally depends on identification of sources and routes of transmission. Since *Campylobacter* are ubiquitous in the environment and most cases are sporadic, identifying sources is difficult. Source attribution methods require isolation of strains from reservoirs to compare *Campylobacter* strain diversity in foods and animals to that in human infections. Beef, sheep and pig meat have a lower prevalence of *Campylobacter* contamination than chicken meat (<5% to 14%),[19-21] but a higher prevalence is found in animal offal such as liver,[22] thus making offal a valuable source of host-associated strains of *Campylobacter* in low-prevalence meats.

STUDY RATIONALE

In the United States, evidence from case-control studies have led to policy change, including changes to chicken slaughtering techniques. The incidence of human *Campylobacter* infection has declined in the US since this policy was introduced in 1997.[23] More recently, evidence from source attribution analyses in New Zealand has led to the development of poultry production policies and practices aimed at reducing the risk of *Campylobacter* transmission via poultry food products.[24] New Zealand has seen a 74% reduction in the number of campylobacteriosis cases attributed to poultry in the region, as well as a 54% reduction in cases overall.[25]

Source attribution modelling enables us to determine which foods and animals are the most likely sources of infection with each *Campylobacter* strain type, and the proportion of cases attributed to each source. This can be done with simple proportional similarity index (PSI) calculations, or by using more complex models.[24] Source attribution also allows for human campylobacteriosis cases to be grouped by potential source, increasing the specificity of risk factor analyses. These source-assigned analyses combine the epidemiological information gained through the traditional case-control study with source attribution modelling to provide greater explanatory power to investigate locally-relevant risk factors.

OBJECTIVES

This study aims to:

1. Identify dietary, environmental and behavioural risk factors for *Campylobacter* infection in Australia
2. Strengthen the epidemiological evidence for previously identified risk factors in Australia
3. Identify strain-specific risk factors for infection using Whole Genome Sequencing (WGS) data from case isolates

HYPOTHESES

We will test several hypotheses regarding specific risk factors for *Campylobacter* infection in Australia. The hypotheses are based on exposures which have previously been identified as risk factors for *Campylobacter* infection in Australia as well as internationally.

We hypothesise that:

1. Persons who consume undercooked meats, particularly chicken, are at increased risk of infection.
2. Persons who consume offal are at increased risk of infection.
3. Persons who own companion animals (especially puppies) are at increased risk of infection.
4. Poor food hygiene and handling practices in the home increase the risk of infection.
5. Most human infections will be attributed to consumption of chicken meat.
6. There will be a high level of genetic diversity amongst *Campylobacter* strains.

STUDY DESIGN

We will conduct a case-control study including genomic testing over a two-year period in three sentinel sites: the state of Queensland (QLD), the Australian Capital Territory (ACT), and Hunter New England (HNE) region of New South Wales (Figure 1). Sporadic cases of culture-positive *Campylobacter* infection will be identified either through state notifiable disease registers, from local pathology service databases or local notification databases. An isolate from each case will be paired with epidemiological data from the case interview. One control will be recruited for each case who participates in the study, with trained interviewers conducting telephone interviews with both cases and controls. Participants will be interviewed using a questionnaire that has been specifically designed to collect information on known potential risk factors. This questionnaire will include a selection of questions being validated in a separate study (Liana Varrone, Validation of questions designed for gastroenteritis investigation). For cases, the questions will cover the seven days prior to the onset of illness, while controls will be questioned on the seven days prior to interview. Meanwhile, *Campylobacter* isolates will also be collected from food and animal samples. All human and non-human isolates will undergo whole genome sequencing for comparison in source attribution modelling. Data for this study will be collected from 1st March 2017 to 1st March 2019.

Figure 1. Map of Australian states and territories, showing the Hunter New England region.

Patient and public involvement

To develop the study, we engaged state and territory health departments, food safety agencies and industry to establish research questions and methods. The process involved a dedicated workshop, followed by teleconferences and an iterative process of drafting study documentation. We also established a reference panel, which includes representatives from senior levels of government and industry bodies. No patients or other members of the public were involved in the development of this study.

STUDY POPULATION

The three sentinel sites cover a population of approximately 6.1 million people. Based on notification and diagnostic pathology data, we expect approximately 8,650 *Campylobacter* cases to be notified across these sites during the study period.

DEFINITION AND SELECTION OF CASES

Case definition

We define a case as a person from any of the three participating sites with a recent history of acute diarrhoea and a culture-positive stool result for *Campylobacter*.

SAMPLE SIZE

We used risk factor prevalence data from a previous national *Campylobacter* case-control study in 2001/2002 to estimate sample size for this study.[26] For example, the prevalence of chicken consumption among controls in 2001/2002 was 80%. A sample size of approximately 1,040 subjects (520 cases; 520 controls) would enable the study to detect an association between chicken consumption and illness with an odds ratio of 1.6, at 80% power and $\alpha = 0.05$, as reported in the previous study. Sample size estimates for other potential risk factors are listed in Table 1.

Table 1. Sample size estimates for an unmatched case-control study

Risk factor	Prevalence of exposure among controls (%)	Prevalence of exposure among cases (%)	Odds ratio	No. of required study subjects
Beef	78	85	1.6	960
Pork	52	60	1.4	1130
Lamb	42	50	1.4	1120
Chicken	80	87	1.6	1040
Offal	2.0	5.0	2.6	1154
Puppies	2.1	5.4	2.7	1040

80% power and $\alpha = 0.05$

From these calculations, we estimate that a study of 1,200 subjects (600 cases; 600 controls) will adequately detect significant associations of these magnitudes for potential risk factors of interest.

Queensland and Hunter New England sites will each enrol at least 250 cases into the study, while ACT will enrol at least 100 cases. Based on the previous Australian case-control study,[26] we expect approximately 80% of selected notified cases to be eligible and participate in the study (Table 2).

Table 2. Sampling method for cases in each site

State	Expected number of notified cases during study period	Estimated cases from participating pathology laboratory	Culture +ve cases	Sequential sampling of notified cases	Total no. of cases	Expected no. to be recruited (~ 80% participation rate)
QLD	7000	2800 (40%)	1260 (45% in QLD)	Select every 4th case	315	250
ACT	600	130	130	Include all notified cases	130	100
NSW (Hunter New England)	~1050	313	313	Include all notified cases	313	250
Total	**8650**	**3243**	**1703**		**758**	**600**

In Queensland, we will obtain cases from one private pathology provider reporting approximately 40% of the state's *Campylobacter* notifications. We estimate that this provider will notify 2,800 cases during the study period with an estimated 45% of these being culture-positive (1,260 notified cases). In ACT, approximately 600 *Campylobacter* notifications are expected during the study period; 130 are expected from the participating pathology laboratory. In Hunter New England, approximately 1,050 *Campylobacter* notifications are expected during the study period; 313 of these notifications will be from the participating pathology laboratory.

Enrolment of Cases

We will enrol all cases who meet the eligibility criteria (Table 3). Each site will check for new notifications of culture-positive *Campylobacter* infection daily, with only culture-positive *Campylobacter* cases eligible for this study. If a case refuses to participate in the study, we will select a subsequent case for inclusion. Enrolment of cases will depend on consent from the patient, or in the event of a child aged less than 18 years, consent from either one of the parents or the child's guardian. We will interview cases as soon as possible by telephone, preferably within two weeks of notification from the laboratory. It will be at the parent's or guardian's discretion as to whether a

child aged between 15 and 17 years is interviewed directly. The parent or guardian will be interviewed for cases aged less than 15 years.

Table 3. Eligibility criteria for cases and controls

Criteria	Cases	Controls
Had diarrhoea (≥3 loose bowel movements in 24hrs)	Include	Exclude
Known date of illness onset	Include	N/A
Household members positive for *Campylobacter* in 4 weeks prior to onset of illness	Exclude	Exclude (4 weeks prior to interview date)
Household members experiencing diarrhoea in 4 weeks prior to onset of illness	Exclude	Exclude (4 weeks prior to interview date)
Travelled outside of Australia in 2 weeks prior to onset of illness	Exclude	Exclude (2 weeks prior to interview date)
Travelled interstate for the entire 2 weeks prior to onset of illness	Exclude	Exclude (2 weeks prior to interview date)
Can't speak English	Exclude	Exclude
Not able to answer questions for some other reason (e.g. intellectually disabled)	Exclude	Exclude
Not contactable after 6 telephone attempts	Exclude	Exclude
Live outside the catchment areas	Exclude	Exclude
Do not have a telephone number available for their primary residence, or a mobile phone	Exclude	Exclude
An enteric pathogen other than *Campylobacter* was isolated/detected in their stool (excluding *Blastocystis hominis* and *Dientamoeba fragilis*)	Exclude	N/A

DEFINITION AND SELECTION OF CONTROLS

We will recruit controls from notified cases of influenza, frequency matched by sex, age group and geographical area of residence by Statistical Area Level 4 (SA4). These controls will be selected with a delay of at least six months from their influenza infection to ensure that controls have returned to eating their customary diet.

Each participating site (QLD, ACT or HNE) will establish a database of controls (previous influenza cases). All cases of influenza notified to the health department in each site between 1st January and 31st December 2017 will be entered into this control database. The age bands are 0-4 years, 5-14 years, 15-34 years, 35-54 years, 55-74 years, and ≥75 years. An appropriate control will be randomly selected from the database within 30 days of interview of the notified case.

Case and control recruitment

Interviewers trained in computer-assisted telephone interviewing (CATI) will conduct telephone interviews. A maximum of six attempts will be made to contact any one case or control, with no more than three attempts in any one day. Three calls will be attempted between 9:00am and 3:59pm, and three attempts between 4:00pm and 8:00pm. A text message will be sent to the potential participant after three failed call attempts, indicating that Public Health is trying to contact them. This protocol will be continued until the person is enrolled or excluded.

QUESTIONNAIRES

We will use specific case and control questionnaires for all participants (see Appendix 1). Cases will be asked additional questions about the clinical course of their illness and treatment. Interviewers will ask identical questions regarding exposures such as foods consumed, dining locations, water sources, domestic food handling techniques and exposure to animals of cases and controls. Questions on foods consumed, dining locations, water consumed, animal and pet exposures will be asked based on a seven-day history. Questions on international travel will be asked based on a two-week history. Antibiotic and antacid consumption, immunosuppressive treatment and household history of diarrhoea will be based on a four-week history. Questions on food handling and general kitchen practices will be based on usual practices rather than recent history. Demographic information will be collected from cases and controls. Contact information required to conduct interviews will be stored in a password-protected Excel document with only those needing to contact individuals given access. Piloted questionnaires were modified to remove repetitions, improve clarity, and to ensure that interviews could be conducted within 20 minutes.

DATA HANDLING & RISK FACTOR ANALYSIS

We will undertake descriptive reporting of campylobacteriosis incidence by person, place and time. We will also describe the severity of symptoms, treatment, and burden of illness.

Risk factor analysis will involve the examination of two-by-two contingency tables with chi square or exact tests to determine the presence of univariable associations between variables and disease. To measure the strength of an association, we will estimate odds ratios and calculate 95% confidence

intervals in a univariable analysis, followed by multivariable logistic regression modelling to adjust for potential confounders. Risk factors selected for inclusion in the regression model will include age, season and geographic area, variables with a significant univariable association with disease, and variables with a P-value ≤0.25 that are biologically plausible and of interest to the research team.

LABORATORY ANALYSES

Human samples

As outlined in Table 2, it is expected that 250 human isolates from Hunter New England, 250 from Queensland, 100 from Victoria and 100 from ACT will be sequenced. The initial isolation and confirmation of *Campylobacter* infection will be performed locally in each State/Territory. Only samples with a pure and viable culture will undergo WGS.

Animal and food samples

We will collect samples from chicken meat (covering the two production methods of continually housed and free range/housed), beef, lamb, pork, and from pet dogs. Given low prevalence of *Campylobacter* in meats other than chicken, samples will be collected from offal (preferably liver) from bovine, ovine and porcine sources to ensure sufficient positive samples are obtained for the study. Given the rising importance of chicken liver pate as a source of outbreaks in Australia,[27] chicken offal will also be sampled. Sample sizes by source are based on data from two states to ensure 50 positive samples per food source, and 30 samples in companion animals (Table 4). We will also contact veterinary clinics and teaching hospitals to ensure sufficient *Campylobacter*-positive samples from dogs. Water samples have been omitted from the genomic aspect of this study due to logistical constraints in sampling untreated water sources across the large geographical area involved in this study, and the complexity of designing an appropriate sampling frame. As there is a lack of evidence implicating municipal drinking water as sources of *Campylobacter* infection in Australia[1 26] we excluded water sampling from this study.

Table 4. Sampling to ensure 50 isolates per food source and 30 isolates from companion animals

	Foods						Animals	
	Chicken			**Beef**	**Lamb**	**Pork**	**Dogs**	**Total**
	Continually housed	*Free-range*	*Offal*	*Offal*	*Offal*	*Offal*		
Assumed prevalence	0.7	0.7	0.7	0.14	0.6	0.22	0.2	
Samples required	72	72	72	286	100	272	150	**1041**
Positive isolates	50	50	50	40	60	60	30	**330**

The initial isolation and confirmation of *Campylobacter* will be performed locally at laboratories in each State/Territory, with isolates forwarded to the Microbiological Diagnostic Unit Public Health Laboratory for WGS, except Queensland isolates which will be sequenced at Queensland Health. To detect seasonal and temporal variation in *Campylobacter* genetic types, 1041 non-human samples (estimated to produce 330 *Campylobacter* isolates) will be collected over a period of one year in Queensland, and two years in New South Wales. To assess latitudinal variation in chicken meat samples across eastern Australia, 105 chicken samples (70 chicken meat and 35 chicken offal) will be collected over a six-month period in Victoria. Food samples will be collected monthly from retail premises, using protocols from surveys undertaken in 2014 by partner organisations, with a pilot of 30 isolates in Queensland.

We will also collect an additional 20-30 human isolates from four additional Australian jurisdictions not participating in this case-control study to undergo WGS. This will be done over a two-month period that overlaps with the case-control study sample collection, and is planned to help inform the generalisability of the case-control study.

SEQUENCING AND SEQUENCE DATA PROCESSING

Campylobacter isolates selected for sequencing will be repurified on solid medium and a single colony selected for preparation of genomic DNA. A sequencing library will be prepared from the genomic DNA for sequencing on the Illumina sequencing platform (MiSeq or NextSeq). A sample of the selected colony will be regrown and cryopreserved (resuspended in liquid medium supplemented with 10% Glycerol and stored at -80^{o}C). In some cases, *Campylobacter* enrichment cultures will be cryopreserved to enable future investigation of the genetic diversity of *Campylobacters* present. The short-read, paired end dataset produced by the Illumina Instrument from the genomic DNA of each isolate will be processed to produce a draft genome sequence for the isolate using a *de novo* assembler such as MEGAHIT.[28] The draft genome sequence will be annotated using Prokka.[29] We will use the draft genome sequence to perform the initial sub-species classification by deriving a multilocus sequence type (MLST) using the "*Campylobacter jejuni/coli*" typing scheme (pubmlst.org). Again, using the draft genome sequence, further typing e.g. virulence factors (http://www.mgc.ac.cn/VFs/) or antimicrobial resistance genotype (https://cge.cbs.dtu.dk/services/ResFinder/) will be performed using Abricate (https://github.com/tseemann/abricate). We will perform comparative genomics to examine the genetic relationships between selected subgroups of isolates in more detail using Nullarbor (https://github.com/tseemann/nullarbor).

SOURCE ATTRIBUTION MODELLING

We will analyse the epidemiological data within designated MLST groups or other typing groups derived from the genomic sequence data. Source attribution modelling and source-assigned analyses will be conducted.

Source attribution models combine typing data from isolates from food, animal and humans to estimate the proportion of human infections that can be attributed to animal and food reservoirs.[30] [31] Once inferred MLSTs have been ascertained, the proportional similarity index[25] will be used to assess similarities by source. We will then undertake source attribution analyses by adapting the asymmetric island model which has previously been applied to MLST data[25] [32] using Markov Chain Monte Carlo (MCMC) methods[33] implemented using the free software WinBUGS.[34] These methods will first be applied to MLST data extracted from whole genome sequences (the aforementioned "inferred MLSTs"), and then compared to structured phylogenetic modelling approaches[35] [36] that provide scope to infer inter-host transmission.

We will then group cases according to putative source based on these source attribution methods.[37] For example, all isolates attributed to chicken will be grouped together, regardless of differing strains. These cases attributed to chicken will then be compared to all controls in a risk factor analysis to produce a source-assigned analysis.

SPATIAL CLUSTERS AND TEMPORAL TRENDS

We will use newly-designated WGS-based MLSTs to assess heterogeneity in isolates from food sources and companion animals in Queensland and New South Wales, and in isolates from chicken meat and humans across Queensland, New South Wales, Victoria and ACT. A two-year sampling framework in New South Wales, one year of sampling in Queensland, and previous survey work in these states will allow us to assess the extent of seasonal and temporal trends. Postcode-level data associated with human illnesses will be used to detect space-time clusters using a scan statistic implemented in the free software SaTScan, at the Statistical Area 1 level.[38] We will use a retrospective space-time permutation model to detect high risk clusters by comparing the observed number of illnesses to the expected number in that geographic zone and time-period.[39]

STUDY LINKAGES AND COLLABORATIONS

The CampySource Project Team comprises three working groups and a reference panel. The working groups focus on: food and animal sampling, epidemiology and modelling, and genomics. The reference panel includes expert representatives from government and industry.

The study is supported by the following partner organisations: the Australian National University, Massey University, University of Melbourne, Queensland Health, Queensland Health Forensic and Scientific Services, New South Wales Health, Hunter New England Health, Victorian Department of Health and Human Services, Food Standards Australia New Zealand, Commonwealth Department of Health and AgriFutures Australia – Chicken Meat Program.

CampySource is also supported by collaboration with the following organisations: ACT Health, Sullivan Nicolaides Pathology, University of Queensland, Primary Industries and Regions South Australia, Department of Health and Human Services Tasmania, Meat and Livestock Australia, and New Zealand Ministry for Primary Industries.

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While undertaking studies, LV is supported through an Australian Government Research Training Program (RTP) Scholarship.

DECLARATIONS

Ethics approval and consent to participate

Informed Consent

A suitably trained interviewer will inform potential participants about the purpose, methods and demands of the study. We will obtain verbal consent from all study participants or their guardians.

Persons aged 18 years and older will be interviewed following informed consent. It will be at the parent's or guardian's discretion as to whether a child aged between 15 and 18 years is interviewed directly, following informed parental/guardian consent. Parents/guardians will be interviewed for cases aged less than 15 years, after informed consent is obtained.

Confidentiality

All information and identifiers will be kept confidential. Names and personal identifiers will be collected and entered into computer records but will be password protected. No personal identifiers will be included in any published materials relating to this study. All hard copy questionnaires containing patient identifiers will be stored in locked filing cabinets in a secure location to which only study investigators and interviewers will have access.

Risks and Benefits

Participants will be informed there are no individual benefits associated with the study and that participation is voluntary. Failure to participate or a withdrawal of participation will not affect any future treatment. There is also no risk to the patient, and the only cost is time spent – approximately 20 minutes – being interviewed. They may refuse to answer any of the questions or stop at any time.

Animal Ethics

All procedures involving live animals will be performed in accordance with a protocol approved by the University of Melbourne's Animal Ethics Committee (ethics ID: 1714156).

Consent for publication

Not applicable.

Availability of data and materials

The Illumina read sets produced as part of this study will be published at INSDC (Sequence Read Archive (DDJB/NCBI) or the European Nucleotide Archive (EMBL-EBI))

Competing interests

No authors have any competing interests to declare.

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Author contributions

MDK conceived the original idea for this study. All authors contributed to the study design and analysis plan. LV and RJS wrote the first draft with contributions from all authors. LF was heavily involved in determining timing and logistics in and between all sites. KL assisted in questionnaire design and flow. DB developed the bioinformatics analysis protocol. LV, RJS, LS, MDK and KG were involved in multiple revisions. The final version of the manuscript was approved by all authors.

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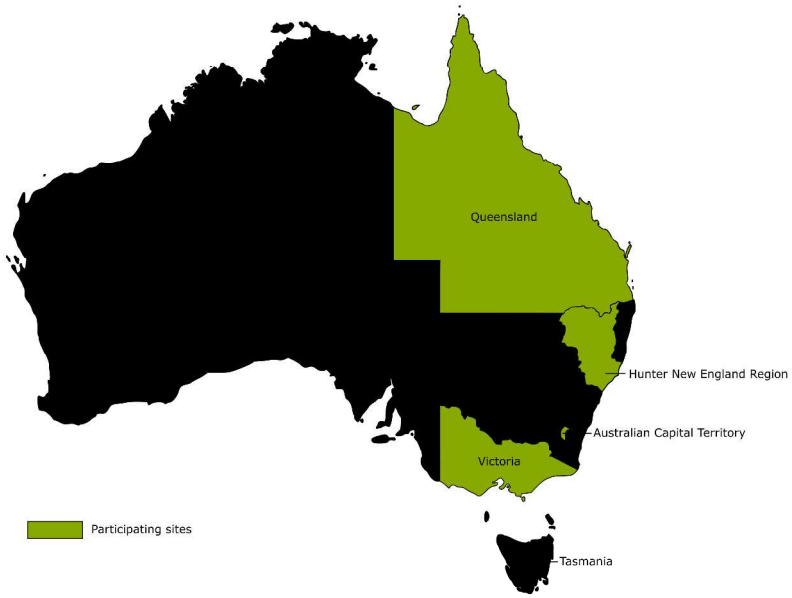

Figure 1. Map of Australian states and territories, showing the Hunter New England region.

Refusal	☐
Ineligible	☐
ID Number	☐☐☐☐☐☐
Interview Date	☐☐☐☐☐☐
Interview Start Time	_____
Data entered	☐
Date	☐☐☐☐☐☐
Data checked	☐
Date	☐☐☐☐☐☐

Source Attribution of *Campylobacter* in Australia Study

Case Questionnaire

INTRODUCTION

Interviewer Note:	If case is less than 15 years of age you will need to speak to parent or guardian most familiar with the eating habits of the child. If case is aged between 15–17 years you will need to obtain parent or guardian consent prior to interview. Please note that for subjects under the age of 15 years, questions relate to the case, not the person being interviewed unless specified in the body of the questionnaire.

"Hello, my name is <Interviewers Name> and I am calling on behalf of [Queensland Health / ACT Health / Hunter New England Public Health Unit]."
"May I please speak with <name of case> or <name of case's mother/father>?"

Interviewer Note:	When the case comes to the phone then repeat the introduction and proceed with the explanatory statement. If the case is unavailable then arrange an alternative time for the interview

"The Australian National University in conjunction with the [state health department] is conducting a study of *Campylobacter* infection in Australia to identify possible causes. *Campylobacter* infections are notifiable to health departments in each state and territory throughout Australia. We understand that you recently experienced an illness due to the *Campylobacter* bacteria. We would like to find out more about your *Campylobacter* illness. Your participation is voluntary, all responses are confidential and if there are any questions you do not wish to answer, just say so."
"Would you be prepared to answer some questions about your illness and activities prior to your illness? The questions will take approximately 20 minutes." Yes ☐ No ☐

"In this study, we will collect information on foods you ate and activities you undertook prior to your illness. Your participation is voluntary and you can stop at any time. No individual information will be presented in any reports or presentations. Partners in this research will comply with the Australian Privacy Act 1988. An information sheet about this research project is available on the ANU website.

"Would you like me to send you a copy or provide you with the link?" Yes ☐ No ☐

If yes, "Could you give me an address/email?" _____

"This study has been approved by the Australian National University Ethics Committee (Protocol 2016/426). If you have any concerns please direct them to
Human Research Ethics Committee
Research Services Office, Chancellery 10B
The Australian National University, ACT 2601
Tel: 6125 7945 Fax: 6125 4807
Email: Human.Ethics.Officer@anu.edu.au"

"Do you have any further questions about the project?" Yes ☐ No ☐

Do you agree to participate in the project?	**Yes** ☐	**No** ☐

"Do you have the time right now to answer these questions?"

If NO, arrange an alternative time to phone back to conduct the interview
If YES, continue

1. ELIGIBILITY QUESTIONS

"Because I will be asking about specific dates around the time of your illness, it may be helpful for you to have a calendar or diary in front of you. Do you need a few minutes to get these?"

Yes, I will get one no ☐
No, I already have one with me ☐
Don't have access to a calendar ☐

The first few questions we'll be asking you are about some symptoms that are associated with [your/their] illness.

1. For the purposes of this study, we define diarrhoea as 3 or more loose stools or bowel movements in any 24-hour period. When you had your *Campylobacter* infection, did you have diarrhoea?

Yes ☐	1	**Go to Q2**
No ☐	2	**Check ineligible box then END INTERVIEW**
Don't know/Not sure ☐	7	**Check ineligible box then END INTERVIEW**

1a During this diarrhoeal illness, what was the maximum number of stools or bowel movements you had in any 24 hour period?

0-2 ☐	1	**Check ineligible box then END INTERVIEW**
3-5 ☐	2	*(If response = '0–2', then recode Q.1 as = '2')*
6-10 ☐	3	
11-20 ☐	4	
More than 20 ☐	5	
Don't know/Not sure ☐	7	

2. For how many days did your diarrhoea last? DAYS

Don't know/Not sure ☐ 77

CALCULATE PRIOR TO INTERVIEW

Date stool specimen collected ☐☐ ☐☐ ☐☐
Day Month Year

3. Could you please let me know what the date was when your diarrhoea began?

☐☐ ☐☐ ☐☐
Day Month Year

(If person is unsure of date then prompt with date of stool specimen)

Don't know/Not sure ☐ 7 **Check ineligible box then END INTERVIEW**

I will now just enter a couple of other dates that we will be talking about throughout the interview. I won't be a moment....

Interviewer Note:	Refer to your calendar to determine the interval from DATE 4 WEEKS BEFORE DIARRHOEA BEGAN to DATE 1 DAY BEFORE DIARRHOEA BEGAN.

4. **In the 4 weeks before your illness began, that is from <DATE 4 WEEKS BEFORE DIARRHOEA> through <DATE 1 DAY BEFORE DIARRHOEA BEGAN>, did anyone else in your household test positive for *Campylobacter*?**

Yes ..... ☐ 1 **Check ineligible box then END INTERVIEW**
No ..... ☐ 2
Don't know/Not sure ..... ☐ 7

5. **In the 4 weeks before your illness began, that is from <DATE 4 WEEKS BEFORE DIARRHOEA> through <DATE 1 DAY BEFORE DIARRHOEA BEGAN>, did anyone else in your household have diarrhoea?**

Yes ..... ☐ 1 **Check ineligible box then END INTERVIEW**
No ..... ☐ 2
Don't know/Not sure ..... ☐ 7

Interviewer Note:	Refer to your calendar to determine the interval from DATE 2 WEEKS BEFORE DIARRHOEA BEGAN to DATE 1 DAY BEFORE DIARRHOEA BEGAN.

6. **In the 2 weeks before your illness began, that is from <DATE 2 WEEKS BEFORE DIARRHOEA> through <DATE 1 DAY BEFORE DIARRHOEA BEGAN>, did you travel overseas or interstate?**

INTERVIEWER NOTE:
IF participant answers "yes",
1. Clarify if the travel was overseas or interstate
2. If travel was interstate:
Clarify the length of time spent interstate in the time period just mentioned

Options to select:
A. If the participant has travelled overseas or spent the whole two weeks interstate: (Select option Yes)
B. If the participant has travelled interstate only for a portion of the time: (Select option No)

Yes..... ☐ 1 **Check ineligible box then END INTERVIEW**
No..... ☐ 2
Don't know/Not sure..... ☐ 7

2. HEALTH QUESTIONS

7. During this illness, did you have any of the following symptoms?

		Yes	No	DK/NS
a.	Fever	☐ 1	☐ 2	☐ 7
b.	Vomiting	☐ 1	☐ 2	☐ 7
c.	Stomach cramps	☐ 1	☐ 2	☐ 7
d.	Blood in your stool	☐ 1	☐ 2	☐ 7
e.	Nausea	☐ 1	☐ 2	☐ 7
f.	Headache	☐ 1	☐ 2	☐ 7
g.	Muscle/body aches	☐ 1	☐ 2	☐ 7

8. Did you take any antibiotics as a result of this illness?

Yes ☐ 1
No ☐ 2 **Go to Q. 10**
Don't know/Not sure ☐ 7 **Go to Q. 10**

9. What antibiotic(s) were you taking? [Ask person to get tablet bottle, if possible]

Azithromycin ☐
Ciprofloxacin ☐
Norfloxacin ☐
Erythromycin ☐
Doxycycline (also known as Doxy or Vibramycin) ☐
Other (please specify) ☐ Specify(_____)
Don't know/Not sure ☐

10. Were you admitted to hospital overnight because of this illness?

Yes ☐ 1
No ☐ 2 **Go to Q. 12**
Don't know/Not sure ☐ 7 **Go to Q. 12**

11. If yes, **for how many nights were you hospitalised?** ☐ NIGHTS
Don't know/Not sure ☐ 7

Interviewer Note:	Refer to your calendar to determine the interval from DATE 4 WEEKS BEFORE DIARRHOEA BEGAN to DATE 1 DAY BEFORE DIARRHOEA BEGAN.

"For the next few questions, I would like to ask you about events which may have occurred in the 4 weeks before your illness began, so again that's from <DATE 4 WEEKS BEFORE DIARRHOEA BEGAN> to <DATE 1 DAY BEFORE DIARRHOEA BEGAN>."

12. In those 4 weeks, were you taking any antibiotics?

Yes ☐ 1
No ☐ 2 **Go to Q. 14**
Don't know/Not sure ☐ 7 **Go to Q. 14**

Interviewer Note:	If person can't remember the name of the antibiotic(s), check the DK/NS box and leave the space blank.

13. What antibiotic(s) were you taking? [Ask person to get tablet bottle, if possible]

		DK/NS	**What date did you stop taking these?**
a.	Antibiotic 1_____	☐ 7	☐☐ ☐☐ (DD/MM) ☐ 7 **DK/NS**
b.	Antibiotic 2_____	☐ 7	☐☐ ☐☐ (DD/MM) ☐ 7 **DK/NS**
c.	Antibiotic 3_____	☐ 7	☐☐ ☐☐ (DD/MM) ☐ 7 **DK/NS**
d.	Antibiotic 4_____	☐ 7	☐☐ ☐☐ (DD/MM) ☐ 7 **DK/NS**

14. In those 4 weeks, were you taking any regular medication that decreases stomach acid?

Yes.....	☐ 1	
No.....	☐ 2	**Go to Q. 16**
Don't know/Not sure.....	☐ 7	**Go to Q. 16**

15. Did you take any of the following in the 4 weeks prior to illness?

Histamine-2 (H_2) Receptor blocker

		Yes	**No**	**DK/NS**
a.	Zantac (Ranitidine)……	☐ 1	☐ 2	☐ 7
b.	Tagamet (Cimetidine)……	☐ 1	☐ 2	☐ 7
c.	Pepcid (Famotidine)……	☐ 1	☐ 2	☐ 7
d.	Axid (Nizatidine)……	☐ 1	☐ 2	☐ 7

Proton Pump Inhibitor

a.	Losec (Omeprazole)……	☐ 1	☐ 2	☐ 7
b.	Nexium (Esomeprazole)……	☐ 1	☐ 2	☐ 7
c.	Somac (Pantoprazole)……	☐ 1	☐ 2	☐ 7
d.	Pariet (Rabeprazole)……	☐ 1	☐ 2	☐ 7
e.	Zoton (Lansoprazole)……	☐ 1	☐ 2	☐ 7

16. Have you ever been told by a doctor that you have any other long lasting condition or chronic illness in which diarrhoea or vomiting is a major symptom? (e.g. Crohn's disease, irritable bowel syndrome, ulcerative colitis, or stomach or oesophagus problems)

Yes.....	☐ 1	Specify(_____)
No.....	☐ 2	
Don't know/Not sure.....	☐ 7	

17. In the 4 weeks before onset of illness, did you take or receive any of the following?

INTERVIEWER NOTE:
Cyclosporine ("it's an immunosuppressant")

		Yes	No	DK/NS
a.	Prednisone or other steroids not used on your skin……..…	☐ 1	☐ 2	☐ 7
b.	Cyclosporine ……..……..	☐ 1	☐ 2	☐ 7
c.	Chemotherapy……..……..	☐ 1	☐ 2	☐ 7
d.	Radiation therapy……	☐ 1	☐ 2	☐ 7

3. EXPOSURES

Interviewer Note: Refer to your calendar to determine the interval from the DATE 7 DAYS BEFORE DIARRHOEA BEGAN to the DATE 1 DAY BEFORE DIARRHOEA BEGAN

A. WATER

"I'm now going to ask you some questions about water that you consumed in the 7 days before your diarrhoea began, that is from [diarr_7_days_prior] to [diarr_1_day_prior].

18. What is your main source of drinking water at home? (select one only)

INTERVIEWER NOTE:
Only read out options if they're unsure

a.	A rainwater tank…….……	☐ 1
b.	A river or stream ……	☐ 1
c.	A private well, bore hole, or spearpoint……	☐ 1
d.	A carrier or tank truck……	☐ 1
e.	Municipal water supply (tap water)……	☐ 1
f.	Purchased bottle water……..	☐ 1
g.	Other water supply……	☐ 1 Specify (_____)
h.	Don't know/Unsure……	☐ 1

Interviewer Note: If person answered "Yes" to "Municipal water supply" or "Purchased bottle water", **skip to Q.21**

19. Do you usually treat your main source of drinking water before drinking?

If Required PROMPT: Some examples are chlorination, filtration, boiling and UV treatment of the water

Yes…….	☐ 1	Specify (_____)
No…….	☐ 2	**Go to Q. 21**
Don't know/Not sure……...	☐ 7	**Go to Q. 21**

20. Which of the following treatments are in place? (select all that apply)

		Yes	**No**	**DK/NS**
a.	Chlorination……..……	☐ 1	☐ 2	☐ 7
b.	Filtration……..……	☐ 1	☐ 2	☐ 7
c.	Boiling…… ……..…	☐ 1	☐ 2	☐ 7
d.	UV treatment…….…….	☐ 1	☐ 2	☐ 7
e.	Other…… Specify (_____)	☐ 1	☐ 2	☐ 7
f.	Don't know..……..……...	☐ 1	☐ 2	☐ 7

21. Did you <u>drink</u> water from any of the following sources in the 7 days before onset of diarrhoea…?
(Select all that apply)

		Yes		No		DK/NS	
a.	A rainwater tank……..……	☐	1	☐	2	☐	7
b.	A river or stream ……	☐	1	☐	2	☐	7
c.	A private well, bore hole, or spearpoint……	☐	1	☐	2	☐	7
d.	A carrier or tank truck……	☐	1	☐	2	☐	7
e.	Municipal water supply (tap water)…….	☐	1	☐	2	☐	7
f.	Purchased bottle water……..	☐	1	☐	2	☐	7
g.	Other water supply…….	☐	1	☐	2	☐	7

Specify (_____)

B. DINING LOCATIONS

"The next few questions ask about places where you may have eaten food in the 7 days before your illness began. So that is from [diarr_7_days_prior] to through [diarr_1_day_prior]"

22. During this time, did you eat any food prepared outside your home, for example takeaway, restaurant, someone else's home?

Yes	☐ 1	
No	☐ 2	**Go to Q 24**
Don't know/Not sure	☐ 7	**Go to Q 24**

23. Did you eat any food from the following places?

		Yes	**No**	**DK/NS**
a.	Café or restaurant	☐ 1	☐ 2	☐ 7
b.	Home cooked meal at someone else's home	☐ 1	☐ 2	☐ 7
c.	Kebab shop	☐ 1	☐ 2	☐ 7
d.	Other fast food/take away outlet	☐ 1	☐ 2	☐ 7

23a How many meals prepared outside of your home, were eaten during this 7 day period?

1-2 meals	☐ 1
3-4 meals	☐ 2
≥ 5 meals	☐ 3
Don't know/Unsure	☐ 7

C. DAIRY PRODUCTS

"I would now like to ask you about the dairy products you may have eaten in the 7 days before your diarrhoea began."

24. Did you drink any raw/unpasteurised milk or eat any products made from raw/unpasteurised milk?

INTERVIEWER NOTE:
Cold-pressed milk is pasteurised and is not to be included as "raw/unpasteurised".

		Yes	No	DK/NS
a.	Unpasteurised milk……	☐ 1	☐ 2	☐ 7
b.	Other products……	☐ 1	☐ 2	☐ 7
	Specify (_____)			

D. MEAT AND POULTRY

"I will now ask you some questions about meat and poultry that you may have eaten in the 7 days before your diarrhoea began, that is from <DATE 7 DAYS BEFORE DIARRHOEA BEGAN> through <DATE 1 DAY BEFORE DIARRHOEA BEGAN>."

25. During these 7 days, did you eat any of the following deli meats or cold cuts?

	Yes	No	DK/NS
a. Salami/mettwurst ……	☐ 1	☐ 2	☐ 7
b. Cabanossi/cabana/twiggy sticks……	☐ 1	☐ 2	☐ 7
c. Ham/chicken/turkey/beef……..……	☐ 1	☐ 2	☐ 7
d. Devon/frankfurts/cheerios……..…	☐ 1	☐ 2	☐ 7
e. Liverwurst…….……..	☐ 1	☐ 2	☐ 7
f. Other ……..	☐ 1	☐ 2	☐ 7

Specify (_____)

26. During these 7 days, did you eat any pate?

Yes..... ☐ 1
No..... ☐ 2 **Go to Q.29**
Don't know/Not sure..... ☐ 7

27. Was the pate eaten,

Chicken pate..... ☐ 1
Duck pate..... ☐ 2
Pork pate..... ☐ 3
Another type of pate..... ☐ 4 Specify (_____)
Don't know/Not sure..... ☐ 7

28. Was this pate homemade or purchased from a store?

Homemade..... ☐ 1
Store..... ☐ 2
Don't know/Not sure…..... ☐ 7

29. During these 7 days, did you eat any other meat or poultry? Like beef, lamb, chicken etc.

INTERVIEWER NOTE:

This does not include eggs

Yes..... ☐ 1
No..... ☐ 2 **Go to Q.49**
Don't know/Not sure..... ☐ 7

BEEF / VEAL

30. During these 7 days, did you eat any beef or veal?

Yes..... ☐ 1
No..... ☐ 2 **Go to Q. 32**
Don't know/Not sure..... ☐ 7

31. During the 7 days prior, did you eat any of the following beef or veal?

		Yes	No	DK/N	
a.	Minced beef dishes…… *(eg. bolognese sauce, pie, pastie, lasagne, hamburger patties, sausages)*	☐ 1	☐ 2	☐ 7	
b.	Kebabs/souvlaki……	☐ 1	☐ 2	☐ 7	
c.	Offal…… *(eg. tripe, liver, tongue)*	☐ 1	☐ 2	☐ 7	Specify (_____)
d.	Other…… *(eg. casserole, stir fry, steak, fillet, roast, beef strips)*	☐ 1	☐ 2	☐ 7	

PORK

32. During these 7 days, did you eat any pork?

Yes..... ☐ 1 Specify (_____)
No..... ☐ 2 **Go to Q. 34**
Don't know/Not sure..... ☐ 7

33. During the 7 days prior, did you eat any of the following pork?

		Yes	No	DK/N	
a.	Minced pork dishes…… *(eg. bolognese sauce, pie, pastie, lasagne, hamburger patties, sausages)*	☐ 1	☐ 2	☐ 7	
b.	Kebabs/souvlaki……	☐ 1	☐ 2	☐ 7	
c.	Offal…… *(eg. tripe, liver, tongue)*	☐ 1	☐ 2	☐ 7	Specify (_____)
d.	Other…… *(eg. casserole, stir fry, steak, fillet, roast, pork strips)*	☐ 1	☐ 2	☐ 7	

LAMB

34. During these 7 days, did you eat any lamb/mutton?

Yes..... ☐ 1 Specify (_____)
No..... ☐ 2 **Go to Q. 36**
Don't know/Not sure..... ☐ 7

35. During the 7 days prior, did you eat any of the following lamb/mutton?

		Yes	No	DK/N	
a.	Minced lamb/mutton dishes…… *(eg. bolognese sauce, pie, pastie, lasagne, hamburger patties, sausages)*	☐ 1	☐ 2	☐ 7	
b.	Kebabs/souvlaki……	☐ 1	☐ 2	☐ 7	
c.	Offal…… *(eg. tripe, liver, tongue)*	☐ 1	☐ 2	☐ 7	Specify (_____)
d.	Other…… *(eg. casserole, stir fry, steak, fillet, roast, lamb strips)*	☐ 1	☐ 2	☐ 7	

GAME MEAT

36. During these 7 days, did you eat any game meat like kangaroo, wallaby, venison or similar?

Yes…… ☐ 1 Specify (_____)
No…… ☐ 2
Don't know/Not sure…… ☐ 7

POULTRY

37. How often do you <u>usually</u> consume chicken/poultry meat?

3 or more days per week…… ☐ 1
1-2 days per week…… ☐ 2
Once per fortnight…… ☐ 3
Less often than once per fortnight…… ☐ 4
Never…… ☐ 5 **Go to Q.46**
Don't know/Not sure…… ☐ 7

38. During the 7 days before your illness began, did you eat any chicken or other poultry?

Yes…… ☐ 1
No…… ☐ 2 **Go to Q.46**
Don't know/Not sure…… ☐ 7

39. How many meals did you eat that contained chicken or other poultry in the 7 days prior to onset of diarrhoea?

1-2 meals…… ☐ 1
3-4 meals…… ☐ 2
≥ 5 meals…… ☐ 3
Don't know/Not sure…… ☐ 7

40. Did you consume any chicken or poultry at home?

Yes..... ☐ 1
No..... ☐ 2 **Go to Q.43**
Don't know/Not sure..... ☐ 7

41. Was the chicken or poultry purchased…?
(Select all that apply)

Raw and fresh..... ☐ 1
Raw and frozen..... ☐ 2
Pre-cooked..... ☐ 3
Don't know/Not sure..... ☐ 4

42. How was it stored before consumption…?
(Select all that apply)

INTERVIEWER NOTE:
(On the bench)
This is only to be used if they STORE their meat on the bench, this does not include defrosting their meat on the bench.

In the freezer..... ☐ 1
In the fridge..... ☐ 2
On the bench..... ☐ 3
Don't know/Not sure..... ☐ 4

43. Prior to cooking, was the chicken rinsed or washed under running water ….?

Yes..... ☐ 1
No..... ☐ 2
Don't know/Not sure..... ☐ 7

44. During this did time you eat any of the following cooked meats……..…?

	Yes	No	DK/NS
a. Chicken mince..…… *(including hamburger patties, sausages)*	☐ 1	☐ 2	☐ 7
b. Chicken kebabs……..	☐ 1	☐ 2	☐ 7
c. Chicken pieces with bones…. *(i.e. wings, drumsticks, whole chicken)*	☐ 1	☐ 2	☐ 7
d. Chicken pieces without bones. *(i.e. breast, tenderloins)*	☐ 1	☐ 2	☐ 7
e. Offal……	☐ 1	☐ 2	☐ 7
specify: liver	☐ 1	other _____	
f. Duck……..…	☐ 1	☐ 2	☐ 7
g. Turkey……..…	☐ 1	☐ 2	☐ 7

45. During this time, on how many days did you eat poultry?

INTERVIEWER NOTE:
1. A pate is included
2. Eggs are excluded

Days:_____

46. During this time, on how many days did you eat meat (including poultry)?

INTERVIEWER NOTE:
Pate is included

Days:_____

47. During the 7 days prior to illness, did [you/they] eat any meat product, which was raw, rare or appeared undercooked?

Yes.....	☐ 1	
No.....	☐ 2	**Go to Q.49**
Don't know/Not sure.....	☐ 7	

48. Which of the following meats did [you/they] eat that was undercooked?

		Yes	**No**	**DK/NS**
a.	Chicken/poultry……..	☐ 1	☐ 2	☐ 7
b.	Beef or veal…….	☐ 1	☐ 2	☐ 7
c.	Pork……	☐ 1	☐ 2	☐ 7
d.	Lamb/mutton……	☐ 1	☐ 2	☐ 7
e.	Game meat……	☐ 1	☐ 2	☐ 7
f.	Minced meat items……... *(eg. including sausages, hamburger patties)*	☐ 1	☐ 2	☐ 7
g.	Offal (specify type)_____.	☐ 1	☐ 2	☐ 7
h.	Other meat……..……..…. Specify (_____)	☐ 1	☐ 2	☐ 7

49. How do you *prefer* the following meat to be cooked?

INTERVIEWER NOTE:
Raw: Not cooked at all
Rare: Mostly red
Medium: Pink through out
Well done: Brown through out

INTERVIEWER NOTE:
If participant answers Medium/Rare
select the rarer option.. e.g Rare

		Raw	Rare	Medium	Well done
a.	Chicken/Poultry……	☐ 1	☐ 2	☐ 3	☐ 4
b.	Beef/Veal……..	☐ 1	☐ 2	☐ 3	☐ 4
c.	Pork……	☐ 1	☐ 2	☐ 3	☐ 4
d.	Lamb……..……	☐ 1	☐ 2	☐ 3	☐ 4
e.	Hamburgers……..	☐ 1	☐ 2	☐ 3	☐ 4
f.	Minced meat……..	☐ 1	☐ 2	☐ 3	☐ 4

E. GENERAL KITCHEN PRACTICES

"I will now ask you several questions about the way food is usually prepared in your home. Remember, your participation is voluntary and you do not have to answer any of the questions if you don't want to."

50. How many times per week do you cook for members of your household?

INTERVIEWER NOTE:
This section around food prepared in the home refers to the person answering the survey (not necessarily the case or control)

0.....	1	**Go to Q.63**
1-5.....	2	
>5.....	3	
Don't know/Not sure.....	7	

51. Did you handle or prepare any raw meats in the kitchen in the 7 days before your diarrhoea began?

INTERVIEWER NOTE:
Refers to the person answering the survey

Yes.....	1	
No.....	2	**Go to Q.57**
Don't know/Not sure.....	7	

52. Did you handle or prepare raw chicken meat or chicken offal in the 7 days before your diarrhoea began?

INTERVIEWER NOTE:
Refers to the person answering the survey

Yes.....	1	
No.....	2	
Don't know/Not sure.....	7	

Interviewer Note: If person answered "No" to **both** Q.51 and Q.52 then **skip to Q.57**

53. After a knife is used to cut raw meat or poultry, which of the following options do you usually do?

INTERVIEWER NOTE:
Refers to the person answering the survey

	Continue using the knife as is.....	1	
	Rinse the knife before continuing to cook.....	2	
	Wipe the knife before continuing to cook.....	3	
	Wash the knife with detergent before continuing.....	4	
	Change to another knife.....	5	
	Other.....	6	Specify (_____)
	No one prepares meat	7	**Go to Q. 57**
Don't read	Don't know/Not sure.....	8	

54. After a cutting board is used to cut raw meat or poultry, which of the following options do you usually do?

INTERVIEWER NOTE:
1. Does not matter if water is hot or cold
2. Refers to the person answering the survey

Continue using the cutting board as is…… ☐ 1
Rinse the cutting board before continuing to cook…….. ☐ 2
Wipe the cutting board before continuing to cook…….. ☐ 3
Wash the cutting board with detergent before continuing…….. ☐ 4
Change to another cutting board…… ☐ 5
Other…… ☐ 6

Specify (_____)

Don't read Don't know/Not sure..... ☐ 7

55. After handling raw meat or poultry in the kitchen, which of the following would you usually do before continuing to cook?

INTERVIEWER NOTE:
Refers to the person answering the survey

Wipe hands..…… ☐ 1
Quickly rinse hands under a running tap..…….. ☐ 2
Wash hands with soap and water…… ☐ 3
Other…… ☐ 4

Specify (_____)

Don't do anything about hands…… ☐ 6 **Go to Q.57**
Don't read Don't know/not sure..... ☐ 7

56. After washing hands during food preparation, what would you usually dry your hands on?

INTERVIEWER NOTE:
Refers to the person answering the survey

Paper towel …… ☐ 10
Sponge/cloth……..…… ☐ 11
Tea-towel /hand towel …….. ☐ 12
Apron…..…… ☐ 13
Don't dry hands……..…… ☐ 14
Other……..…… ☐ 15

Specify (_____)

Don't read Don't know/Not sure.....…….. ☐ 77

57. In the past 3 months, has anyone in the household cook meat on a BBQ?

Yes.....…….. ☐ 1
No.....…… ☐ 2 **Go to Q. 59**
Don't know/Not sure…….. ☐ 7 **Go to Q. 59**

58. After cooking on the BBQ, where would the cooked meat most likely be placed?

	Back on the same container……	☐ 1	
	Back on the same container after it has been rinsed with water ……..…	☐ 2	
	Back on the same container after it has been wiped off with a towel…..…….	☐ 3	
	Back on the same container, after the container has been washed with soap and water…..…….	☐ 4	
	On a different container ……..……..	☐ 5	
	Other……...	☐ 6	Specify (_____)
Don't read	Don't know/not sure……	☐ 7	

F. ANIMAL AND PET EXPOSURE

"The next few questions are about contact with animals in the 7 days before your diarrhoea began."

59. During this time, did you keep or care for any of the following animals as pets?

INTERVIEWER NOTE:
Not to include one off contact

		Yes	No	DK/NS	Is any pet less than 6 months old? Yes	No	DK/NS
a.	Cat……..	☐ 1	☐ 2	☐ 7	☐ 1	☐ 2	☐ 7
b.	Dog……..	☐ 1	☐ 2	☐ 7	☐ 1	☐ 2	☐ 7
c.	Chickens……..	☐ 1	☐ 2	☐ 7	☐ 1	☐ 2	☐ 7
d.	Other birds……..	☐ 1	☐ 2	☐ 7	☐ 1	☐ 2	☐ 7
e.	Other ……..……..	☐ 1	☐ 2	☐ 7	Specify (_____)		
f.	Do not keep any pets……	☐ 7 **Go to Q.67**					

Interviewer Note: If person answered No/Don't know to Cat then skip to question 62

60. Do you feed your cat raw meat or bones?

Yes..... ☐ 1 Specify (_____)
No..... ☐ 2 **Go to Q. 62** (eg. chicken, beef, kangaroo, lamb etc.)
Don't know/Not sure..... ☐ 7 **Go to Q. 62**

61. How often does your cat get fed raw meat or bones?

Daily..... ☐ 1
Weekly..... ☐ 2
Monthly..... ☐ 3
Less often..... ☐ 4
Don't know/Unsure..... ☐ 7

Interviewer Note: If person answered No/Don't know to Dog then skip to question 65

62. Do you feed your dog raw meat?

Yes..... ☐ 1 Specify (_____)
No..... ☐ 2 (eg. chicken, beef, kangaroo, lamb etc.)
Don't know/Not sure..... ☐ 7

63. Do you feed your dog raw bones?

Yes..... ☐ 1 Specify (_____)
No..... ☐ 2
Don't know/Not sure..... ☐ 7

Interviewer Note:	If person answered No/Don't know to questions 62-63 then skip to question 65

64. How often does your dog get fed raw meat or bones?

Daily..... ☐ 1
Weekly..... ☐ 2
Monthly..... ☐ 3
Less often..... ☐ 4
Don't know/Not sure..... ☐ 7

65. Did you get any of your pets in the 4 weeks before your diarrhoea began?

Yes..... ☐ 1 Pet(s) (_____)
No..... ☐ 2
Don't know/Not sure..... ☐ 7

66. Were any of your own pets ill with diarrhoea in the 7 days before your diarrhoea began?

Yes..... ☐ 1 Pet(s) (_____)
No..... ☐ 2
Don't know/Not sure..... ☐ 7

67. In the 7 days before your diarrhoea began, did you have contact with household pet faeces or manure (eg. changing litter boxes or picking up pet faeces with a plastic bag)?

Yes..... ☐ 1 Pet(s) (_____)
No..... ☐ 2
Don't know/Not sure..... ☐ 7

68. Do you live on a farm/hobby farm including a property on acreage 5 acres or over?

Yes..... ☐ 1
No..... ☐ 2
Don't know/Not sure..... ☐ 7

69. In the 7 days before your diarrhoea began, did you visit a farm or petting zoo?

Yes..... ☐ 1 Specify (_____)
No..... ☐ 2 (eg. private farm, commercial farm, petting zoo etc.)
Don't know/Not sure..... ☐ 7

4. DEMOGRAPHICS

"I would now like to ask you a few final questions. Remember, your participation is voluntary and you do not have to answer any of the questions if you don't want to."

70. Is any language other than English spoken in your household?

	Yes	☐	1
	No	☐	2
Don't read	Don't know/Not sure	☐	7
Don't read	Refused	☐	9

Specify (_____)

71. Are you of Aboriginal or Torres Strait Islander origin?

	No	☐	1
	Aboriginal	☐	2
	Torres Strait Islander	☐	3
	Both	☐	4
Don't read	Don't know/Not sure	☐	7
Don't read	Refused	☐	9

72. Which of the following places best describe where you live?

	Inner city or urban area	☐	1
	Suburban area	☐	2
	Town	☐	3
	Rural or remote area community	☐	4
	Rural or remote area farm or property	☐	5
Don't read	Don't know/Not sure	☐	7
Don't read	Refused	☐	9

Interviewer Note: See definitions below.

Inner city area:……*housing close to the centre of a major/capital city*
Suburban area:..……*housing area further from the centre of the city, which is characterised by the region being primarily a self-contained residential district.*
Town:……*community over 2000 people*
Rural or remote area community:……*community under 2000*
Rural or remote area farm or property

73. Does your occupation involve any of the following?

Working with raw meat…… *(eg. restaurants, butchery, abattoir etc.)*	☐	11
Working with animals…… *(eg. farmer, zookeeper, vet/nurse etc.)*	☐	12
Other type of occupation……	☐	13
Retired……	☐	14
CASE not of working age……	☐	15
Don't know/Unsure……	☐	17

74. What is the highest level of education reached by <u>anyone</u> in your household?

	Schooling to year 10 or below…..……..	☐ 12
	Secondary school, above year 10……	☐ 13
	Technical or further educational institution…….…. *(eg. TAFE, apprenticeship, college etc.)*	☐ 14
	University degree—Undergraduate …….	☐ 15
	University degree—Postgraduate (Masters, doctorate).	☐ 16
Don't read	Don't know/not sure.....	☐ 7
Don't read	Refused…….....….	☐ 9

"Now I am going to read you a list of income categories. Please stop me when a category best describes your total household income, before taxes, in the last financial year? That is the total figure for all household members."

75. Last year the total income for your household was….?

	Less than $25,000.....…….	☐ 1
	$25,000 to $50,000.....……...	☐ 2
	Between $50,000 and $100,000……..	☐ 3
	Between $100,000 and $150,000……..	☐ 4
	More than $150,000.....……...	☐ 5
Don't read	Don't know/Not sure.....……..	☐ 7
Don't read	Refused.....…..……..	☐ 9

76. As part of this research we are planning to do a follow-up study. Would you be happy for us to contact you in ~6 months' time?"

Yes.....……...	☐ 1	
No.....…….....	☐ 2	**Skip to end of questionnaire**

Interviewer Note: If person answered No to Q 76 then skip to the end of the questionnaire

Details required:

Name:_____

Phone number:_____

Email address:_____

"That's my last question. Thank you very much for your time and cooperation."

COMPLETE AFTER INTERVIEW

Interviewer initials _____

Interview stop time _____

Length of interview _____MINUTES

Respondent recall:

Poor	☐ 1
Fair	☐ 2
Average	☐ 3
Good	☐ 4
Excellent	☐ 5