Private water supplies as a risk factor for *Campylobacter* infection in Aberdeen City and Aberdeenshire

Health Protection Scotland

March 2010

CONTACTS

Dr Alison Smith-Palmer and Dr J Cowden
Health Protection Scotland
Clifton House
Clifton Place
Glasgow

Tel. 0141 300 1163 or 0141 300 1122
Email. Alison.Smith-Palmer@nhs.net
Email john.cowden@nhs.net

Dr Kasia Kazimierczak
Food Standards Agency Scotland
St Magnus House
25 Guild Street
Aberdeen
AB11 6NJ

Tel 01224 285179
Email Kasia.Kazimierczak@foodstandards.gsi.gov.uk

ACKNOWLEDGEMENTS

Health Protection Scotland is grateful to the Food Standards Agency for the financial support for this study and to the Health Protection Team NHS Grampian for their collaboration and support of the study.

We acknowledge the support and contribution of the University of Aberdeen for the water testing component of this study and for providing MLST data from the *Campylobacter* MLST project (S14006) for linkage to epidemiological data in this study and their help in the analysis.

Health Protection Scotland is grateful to the Environmental Health Department, Aberdeenshire Council for their support with this study and finally to all the cases and controls who participated in the study.

CONTENTS

	Contacts	Ii
	Acknowledgements	Iii
	Contents	Iv
	Tables	Ix
	Figures	Xii
	Glossary	Xiii
	Technical Summary	Xiv
	Lay person Summary	Xvi
	CHAPTER 1: INTRODUCTION	
1.1	History of *Campylobacter*	1
1.2	World wide importance of *Campylobacter*	1
1.3	Clinical features of *Campylobacter* infection	2
1.4	Epidemiology of *Campylobacter* in Scotland	2
1.5	Epidemiology and Multilocus Sequence Typing (MLST) of *Campylobacter* in Scotland	4
1.6	Variation in rates of infection across Scotland	5
1.7	Risk factors for *Campylobacter* infection	5
1.8	Private water supplies in Scotland	8
	CHAPTER 2: AIMS	
2.1	Primary aim	10
2.2	Secondary aims	10
	CHAPTER 3: METHODS	
3.1	Overview of study methodology	11
3.2	Ethics Approval	11
3.3	Sample size	11
3.4	Cases	11
3.5	Controls	12
3.6	Water testing component of study	13
3.7	Amendment to study during second year of the study	14
3.8	MLST Study (FSAS project S14006)	15
3.9	Details of private water supplies	15
3.10	Other data sources used	15
3.11	Data storage	16
3.12	Pilot study and study dates	16
3.13	De-duplication and cleaning of the database	16
3.14	Data analysis	17

3.14.1	Odds ratios, adjusted odds ratios	17
3.14.2	Associations between exposure factors, symptoms and hospitalisation	18
3.14.3	Relationship between Clonal Complexes and categorical variables using Pearson Chi-square and Likelihood ratio chi-square	18
3.14.4	Differences between exposed *versus* unexposed cases in composition and host attribution of their *Campylobacter* strains	18
	CHAPTER 4: EPIDMEIOLOGICAL RESULTS	
4.1	Participation in the study	20
4.1.1	Total number of questionnaires returned to HPS	20
4.1.2	Cases and controls who declined to participate	21
4.1.3	Undeliverable questionnaires	21
4.1.4	Participation rates among cases	21
4.1.5	Participation rates among controls	21
4.2	Characteristics of cases and controls	23
4.2.1	Age distribution of cases and controls	23
4.2.2	Rates per 100,000 for cases participating in the study and total cases reported to NHS Grampian	24
4.2.3	Residence of cases and controls and population of the study area	26
4.2.4	Deprivation category of cases and controls	27
4.2.5	Comparison to the population of the study area and Scotland	27
4.2.6	Occupation group of cases and controls	28
4.2.7	Year and month of participating in the study	29
4.3	Length of time between onset, completing the questionnaire and completeness of the questionnaire	30
4.3.1	Length of time between onset and completing questionnaire for cases	30
4.3.2	Association between completeness of questionnaire, time between onset and completing questionnaire	31
4.4	Clinical presentation of infection	32
4.4.1	Symptoms reported by cases and controls	32
4.4.2	Admission to hospital	33
4.4.3	Duration of illness	33
4.5	Other members of the same household reported with similar symptoms	36
4.5.1	At least one other member of the same household reported with similar symptoms	36
4.5.2	Two or more laboratory confirmed cases in the same household	36
4.6	Exposure differences between cases and controls	36
4.7.1	Medicines taken regularly by cases and controls	37
4.7.2	Association between medicines taken regularly by cases and admission to hospital	37

4.8.1	Travel outside the study area	39
4.8.2	Locations associated with travel outside the study area	39
4.8.3	Association between travel outside the study area and month of participating in the study	40
4.8.4	Association between travel outside the study area an reporting other members of the same household to be ill with similar symptoms	41
4.9	Animal contact	44
4.9.1	Pet animals	44
4.9.2	Types of pet	44
4.9.3	Pets reported to be ill with diarrhoea or vomiting	44
4.9.4	Contact with farm animals	45
4.10.	Eating food outside the home	46
4.10.1	Eating chicken outside the home	46
4.11	Consumption of foods prepared at home	47
4.11.1	Eating chicken prepared at home	47
4.11.2	Eating red meat prepared at home	47
4.11.3	Consumption of cooked meat at home	47
4.11.4	Consumption of salads or raw vegetables at home	48
4.11.5	Consumption of shellfish at home	48
4.11.6	Consumption of pre-packed sandwiches	48
4.11.7	Consumption of raw/unpasteurised milk	48
4.11.8	Attending barbecues or picnics	49
4.11.9	Consumption of any pre-packed ready to eat foods	49
4.12	Drinking bottled water	51
4.13	Drinking water from other sources	51
4.14	Consumption of fresh vegetables, salads and fruit washed in tap water at home	51
4.15	Water supply	53
4.15.1	Validation of household water source	53
4.15.2	Comparison of cases and controls on mains and private water supplies	54
4.15.3	Association between water source and month of study	55
4.15.4	Association between water source and local authority area	55
4.15.5	Interaction between water source and overnight stay outside the study area	55
4.15.6	Interaction between water source and contact with farm animals	56
4.15.7	Interaction between water source and drinking bottled water	56
4.15.8	Interaction between water source and taking part in water activities	56
4.15.9	Association between water source and other members in the household with similar symptoms	56
4.15.10	Association between water source and changes in water supply	56
4.16	Description of private water supplies	57

4.17	Amount of tap water consumed by participants	59
4.18	Recreational water activities	60
4.19	Summary of unadjusted odds ratio	61
4.20	Adjusted odds ratios	65
4.21	Associations between exposure factors and clinical symptoms	69
5	**CHAPTER 5: WATER TESTING RESULTS**	
5.1.1	Consenting to participate in the water testing component	71
5.1.2	Water testing by participant	71
5.1.3	Number of days between onset and collection of water samples	73
5.2	Results of microbiological water testing	74
5.2.1	Detection of coliforms	74
5.2.2	Detection of *E.coli*	74
5.2.3	Detection of Enterococci	74
5.2.4	Detection of *Campylobacter*	75
5.3	Differences in microbiological water quality between cases and controls on private water supplies	75
5.4	Relationship between the detection of coliforms in a private water supply and the detection of *E.coli,* Enterococci and *Campylobacter*	77
5.5	Seasonality trends in microbial detection from private water supplies	77
5.6	Relationship between category of private water supply and water testing results	77
5.7	Relationship between treatment of private water supply and water testing results	78
6	**CHAPTER 6: MLST RESULTS**	
6.1	Species, clonal complexes and sequence types isolated	81
6.2	Cases infected with *Campylobacter* on more than one occasion	83
6.3	Cases of *Campylobacter* infection resident at the same address	83
6.4	MLST results for water isolates and clinical cases	84
6.5	Relationship between CC and categorical variables	84
6.5.1	Analysis using Pearson Chi-square and likelihood ratio Chi-square	84
6.5.2	Relationship between CC and month of onset	84
6.5.3	Relationship between CC and overnight stay outside the study area and travel abroad	85
6.5.4	Relationship between CC and contact with farm animals	85
6.5.5	Relationship between CC and eating out	85
6.6	Differences between exposed *versus* unexposed cases, in the ST composition and host attribution of	85

	their *Campylobacter* strains	
7	**CHAPTER 7: DISCUSSION**	
7.1	Study design and participation	92
7.2	Demographics; age, deprivation, rural/urban residence	93
7.3	Clinical presentation	95
7.4	General outbreaks	96
7.5	Others in household with similar illness	96
7.6	Identification of risk factors for *Campylobacter* infection	97
7.7	Travel outside the study area	97
7.8	Use of medication	98
7.9	Animal contact	98
7.10	Consumption of chicken inside and outside the home	99
7.11	Drinking bottled water	100
7.12	Recreational water activities	101
7.13	Private water supplies	102
7.14	Potentially protective variables	104
7.15	Water testing component of the study	105
7.16	MLST study component	110
7.17	Further research	112
7.18	Final conclusions	113
8	**CHAPTER 8: SCIENTIFIC PUBLICATIONS**	
8.1	Papers in preparation	114
8.2	Presentations at scientific and stakeholder meetings	114
	References	115
	Appendix A: Additional results tables	127
	Appendix B: Ethical approval and R&D approval	163
	Appendix C: Copy of case questionnaire and study information leaflet	163
	Appendix D: Report for study S14024, Private water supplies water quality, Aberdeen University	163

TABLES

Table 1	Total questionnaires returned and questionnaires included in study	20
Table 2	Questionnaires included for analysis	20
Table 3	Number of adults and children participating in the study	23
Table 4	Cases and controls resident in Aberdeen City and Aberdeenshire	27
Table 5	Symptoms reported by cases and controls	34
Table 6	Number of symptoms reported by cases and controls	34
Table 7	Symptoms reported by cases admitted to hospital and cases not admitted	35
Table 8	Total number of symptoms reported, for those admitted and not admitted to hospital	35
Table 9	Cases and controls included in analysis for exposure differences	37
Table 10	Adult cases and controls taking Omeprazole (Losec), Cimetidine (Tagamet) or Ranitidine (Zantac)	38
Table 11	Use of antibiotics and admission to hospital	38
Table 12	Use of Omeprazole (Losec), Cimetidine (Tagamet) or Ranitidine (Zantac) and admission to hospital	38
Table 13	Cases and controls with an overnight stay outside the study area	41
Table 14	Cases and controls and country visited	42
Table 15	Comparison of top five countries visited by cases, controls and the top ten countries visited by Scottish population, using data from Travel Trends 2006	43
Table 16	Overnight stay outside the study area and reporting other members of the household to be ill with similar symptoms	44
Table 17	Cases and controls who reported having a pet at home	45
Table 18	Cases and controls who reported any contact with farm animals in the 5 days before onset or completing the questionnaire	45
Table 19	Participants who reported eating food outside the home or from ‘carry out’ facilities	46
Table 20	Participants who reporting consuming chicken while eating outside the home	47

Table 21	Consumption of chicken eaten outside the home, combining those who ate outside the home but did not eat chicken and those who report no eating outside the home	47
Table 22	Consumption of chicken prepared at home	49
Table 23	Consumption of red meat prepared at home	49
Table 24	Consumption of cooked meat at home	49
Table 25	Consumption of salads or raw vegetables (including coleslaw) at home	50
Table 26	Consumption of pre-packed sandwiches	50
Table 27	Consumption of raw/unpasteurised milk (in drink or with cereal)	50
Table 28	Attending barbecues or picnics	50
Table 29	Consumption of any pre-packed ready to eat foods	51
Table 30	Consumption of raw vegetables washed in tap water at home	52
Table 31	Consumption of salads washed in tap water at home	52
Table 32	Consumption of fruit washed in tap water at home	52
Table 33	Answers from the questionnaire, as to sources of household water supply	54
Table 34	Final water supply used for analysis	54
Table 35	Cases and controls on mains or private water supplies	57
Table 36	Water source for those with no overnight stay outside the study area in the 14 days before onset or completing the questionnaire	58
Table 37	Number of cases on mains and private water supplies who reported other members of the same household to be ill with similar symptoms	58
Table 38	Treatment of private water supply	58
Table 39	Number of participants drinking tap water on its own at home	59
Table 40	Summary of unadjusted odds ratio for cases and well controls: Whole study area	61
Table 41	Summary of unadjusted odds ratio for cases and all controls: Whole study area	62
Table 42	Summary of unadjusted odds ratio for cases and well controls: Aberdeenshire only	63
Table 43	Summary of unadjusted odds ratio for cases and all controls: Aberdeenshire only	64
Table 44	Adjusted odds ratio for cases and well controls: Whole study area	65
Table 45	Pairwise statistical association between 10 infection risk exposures and four disease symptoms	70
Table 46	Number of cases and controls consenting to participate in the water testing	72
Table 47	Number of children and adults consenting to participate in the water testing	72
Table 48	Number of cases and controls for whom water	72

	testing conducted	
Table 49	Detection of coliforms in the water sample, by type of supply	75
Table 50	Detection of *E. coli* in the water sample, by type of supply	75
Table 51	Detection of Enterococci in water sample, by type of supply	75
Table 52	Detection of *Campylobacter* in water sample, by type of supply	75
Table 53	Detection of coliforms in private water supplies from cases and controls	76
Table 54	Detection of *E. coli* in private water supplies from cases and controls	76
Table 55	Detection of Enterococci in private water supplies from cases and controls	76
Table 56	Relationship between detection of *Campylobacter,* and coliforms, *E.coli* and Enterococci	77
Table 57	Coliforms detected and category of the private water supply	78
Table 58	*E. coli* detected and the category of the private water supply	78
Table 59	Enterococci detected and the category of the private water supply	78
Table 60	Detection of coliforms in treated and untreated private water supplies	79
Table 61	Detection of *E.coli* in treated and untreated private water supplies	79
Table 62	Detection of Enterococci in treated and untreated private water supplies	80
Table 63	Species of *Campylobacter* isolated	81
Table 64	MLST types for cases resident at the same address	83
Table 65	Comparison between clinical isolate from case and isolated from case's water supply	84
Table 66	Likely source of *Campylobacter* isolated from water	84
Table 67	Comparison of strain composition and source attributions between cases exposed *versus* unexposed to different infection risk exposures, and cases with *versus* without different disease symptoms/outcomes	88
Table 68	*Campylobacter* strains yielding evidence for frequency differences between cases exposed *versus* unexposed to overseas travel and contact with farm animals	89
Table 69	*Campylobacter* strains yielding evidence for frequency differences between symptomatic *versus* asymptomatic cases, and hospitalised *versus* non-hospitalised cases.	90
Table 70	Source attributions of cases exposed *versus* unexposed to chicken consumption from both eating out and chicken prepared in the home	91

FIGURES

Figure 1	Laboratory reports of *Campylobacter* to HPS 1988 to 2007	3
Figure 2	Rates per 100,000 population of reports of *Campylobacter,* 2009	5
Figure 3	Participation rates among controls in the main study by sex and age band	22
Figure 4	Matching frequency for males and females	24
Figure 5	Rates per 100,000 for male *Campylobacter* cases in Grampian NHS Board area and cases participating in the study	25
Figure 6	Rates per 100,000 for female *Campylobacter* cases in Grampian NHS Board area and cases participating in the study	26
Figure 7	Deprivation category for cases and controls	28
Figure 8	Year and month questionnaire was received at HPS	30
Figure 9	Days between onset and completing the questionnaire for cases	32
Figure 10	Percentage of case admitted to hospital by age band	34
Figure 11	Duration of illness for case that provided information on onset date and date when symptoms stopped	35
Figure 12	Percentage of cases and controls who reported an overnight stay outside the study area by study year and month	43
Figure 13	Number of cases and controls with a private water supply by month the questionnaire was received	58
Figure 14	Number of days between onset for cases and collection of the water sample	73
Figure 15	Common Sequence types of *Campylobacter* isolated from cases	82
Figure 16	Common clonal complexes of *Campylobacter* isolated from cases	82

GLOSSARY

Attribution	Inference of the source of human *Campylobacter* infection using strain type
cfu	Colony forming units. Typically a measure of the number of live cells in a sample
Clonal complex (CC)	A group of sequence types whose members are linked to at least one other member by being identical for six of the seven MLST genes
Isolate	A *Campylobacter* culture isolated from a specimen by microbiological methods
MLST	Multilocus Sequence Typing
Sequence Type (ST)	Sequence type of a *Campylobacter* isolate, defined as its allelic profile for a standard set of seven housekeeping genes

TECHNICAL SUMMARY

A case control study was conducted between August 2005 and November 2007, with the primary aim of identifying whether the consumption of water from private water supplies is a risk factor for *Campylobacter* infection in Aberdeenshire and Aberdeen City. Secondary aims were to identify whether private water supplies play a role in the seasonality of *Campylobacter* infection and whether there is an association between particular molecular (MLST) types of *Campylobacter* and private water supplies.

Analysis included epidemiological data from 789 cases and a total of 1898 controls; 1589 of whom were classified as 'well controls' that is they reported no symptoms compatible with *Campylobacter* infection and therefore could not have been unidentified cases.

The study clearly identified the risk associated with private water supplies; the adjusted odds ratio for all cases resident in Aberdeen city and Aberdeenshire compared to well controls was 3.062 (2.056 – 4.562). This is the first time that the private water supplies have been associated with someone becoming a sporadic case of *Campylobacter* infection in Scotland. While there have been previous outbreaks of *Campylobacter* infection in the UK associated with private water supplies, outbreaks account for only a very small proportion of cases, with almost all cases being sporadic. The risks identified in outbreaks are not necessarily the same as those for sporadic cases.

This study found no significant association between the risk of *Campylobacter* infection linked with private water supplies and seasonality ($X^2 = 0.903$, df = 3, p = 0.825). Contamination of private water supplies may be due to a number of factors, including direct contamination of the water by animal faeces or by run off into the water source. As such, contamination may be related to animal grazing and rainfall. Heavy rainfall after a period of dry weather may present a particular risk of run off into private water supplies and as such contamination may be more closely related to weather parameters and farming practices than the season *per se*.

The second component of the study was the microbiological testing of water supplies from cases and controls. Coliforms, *E. coli* and Enterococci were all significantly more likely to be detected from private water supply samples than mains samples. There was little statistical difference in the microbial quality of water from private water supplies belonging to cases than those of controls.

The identification in the study of the risk of *Campylobacter* infection associated with some other risk factors in particular travel (which was defined as an overnight stay outside the study area, and further characterised to that within the UK and abroad) and the consumption of chicken when eaten outside the home was consistent with a number of other studies. Due to the linkage of epidemiological data to molecular typing data, the study identified a significant association between travel abroad and specific clonal complexes and found greater diversity in strain types among cases that had travelled abroad than those that hadn't. The analysis also identified an association with differences in sequence types and clonal complexes for cases with contact with farm animals.

When considering the host attribution of strains as determined in a previous related MLST study (FSA project S14006), only contact with farm animals and private water supplies were associated with differences in the host attribution of strains. Both factors showed cases had strains with higher ruminant and lower chicken attribution. However, there was no evidence that exposure to chicken either eaten out or prepared at home or both was associated with any increased source attribution to chicken.

The study has clearly demonstrated for the first time in Scotland that the consumption of water from private water supplies is a risk factor for *Campylobacter* infection in Aberdeenshire and Aberdeen City and has highlighted the significant difference in the microbial quality of water from private water supplies compared to mains supplies. The study also identified other risk factors for *Campylobacter* infection including travel abroad, and has had the unique opportunity to investigate the epidemiology of *Campylobacter* infection with the inclusion of molecular typing information. Understanding the risk of *Campylobacter* infection associated with private water supplies will hopefully lead to improvements in the water quality from such supplies and a reduction in the risk of *Campylobacter* infection from them.

LAY PERSON SUMMARY

Campylobacter is an important cause of gastrointestinal illness, with 6378 cases reported in Scotland in 2009. The actual number of cases is likely to be in the region of 51000, as only about 1 in 8 of those infected will seek medical attention and have a stool sample submitted for laboratory testing.

Whilst some of the risk factors for *Campylobacter* infection have been established through other studies, in particular overseas travel and eating chicken, these only account for some of the cases and there are many potential risk factors that we do not fully understand. This study investigated the role of private water supplies as a risk factor for *Campylobacter* infection in Aberdeenshire and Aberdeen city.

The study was conducted during August 2005 and November 2007 and collected information from confirmed cases of *Campylobacter* infection and compared this to similar information collected from similar but well people - "controls". The study found that cases were between two and four times more likely to have a private water supply as their home water supply than controls, suggesting that private water supplies can be an important source of *Campylobacter* infection.

Another aspect of the study was to compare the water quality of those with a mains supply and those with a private water supply. The study found that private water supplies were significantly more likely to be contaminated with a range of bacteria including coliforms, *E. coli* and Enterococci than mains water supplies, and that there was very little difference in the quality of private water supplies belonging to cases compared with those of controls.

The findings of this study also supported the findings of other studies in identifying travel abroad as an important risk factor, and also the consumption of chicken when eaten outside the home.

This was one of the first studies to also use the molecular typing of *Campylobacter* to further understand its epidemiology. This aspect of the study showed that both travel abroad and having contact with farm animals were significantly associated with the specific molecular type of *Campylobacter* with which the case was infected. Cases who had travelled abroad had a greater diversity in the molecular types of *Campylobacter* isolated than those who had not.

This study has helped to understand some of the risk factors associated with *Campylobacter* infection, and in particular the risk associated with drinking water from a private water supply. Such an understanding will hopefully help to lead to improvements in the water quality from such supplies and a reduction in the risk of *Campylobacter* infection from them.

CHAPTER 1: INTRODUCTION

1.1 History of *Campylobacter*

The bacteria now classified as *Campylobacter* were formally assigned to the *Vibrio* genus, and were originally recognised at the start of the last century as principally a veterinary pathogen causing septic abortions in sheep and cattle (Acheson & Allos 2001). In 1957, King described the isolation of *Vibrio* from children with diarrhoea (Altekruse *et al* 1999), but it was not until the 1970s and in particular the work of Butzler *et al* (1973) and Skirrow (1977) that the importance of *Campylobacter* as a human pathogen emerged. Today *C. jejuni* and *C. coli* are regarded as the two most important species for human *Campylobacter* infection, accounting for approximately 93% and 7% of *Campylobacter* isolates from humans, respectively (Gillespie *et al* 2002). Other species including *C. upsaliensis* and *C. lari* are occasionally isolated from humans.

1.2 World wide importance of *Campylobacter* infection

Poor hygiene and sanitation and close proximity to animals in developing countries contribute to the easy and frequent acquisition of enteric pathogens including *Campylobacter. Campylobacter* is hyperendemic in developing countries where it is a leading cause of infantile diarrhoea (Coker *et al* 2002). It is the most commonly isolated bacterial pathogen from children under two years of age, but does not appear to be important in adults. In contrast in developed countries it is an important pathogen across all age groups.

Since 1997 *Campylobacter* is recognised as the most frequently reported bacterial cause of infectious intestinal disease in many developed countries (Blaser 1997) including Scotland, however there is considerable variation in rates of infection reported from developed countries. Data from fifteen European countries for 1999 showed rates ranging from 2.9 to 166.8 per 100,000 with a mean of 61 per 100,000 (Takkinen *et al* 2003). However, these figures should be viewed with caution, as direct comparison of the reported incidence between countries is difficult due to differences in the national systems for the reporting of such infections at regional and national levels. In some countries the coverage achieved by the national surveillance centre does not encompass the whole population. Differences also exist in the nature of reporting in terms of voluntary or mandatory, who the notifying partners are (physicians or laboratories), differences in diagnostic techniques at local and reference laboratories and the provision of medical services for the treatment of cases and submission of stool samples. However, these difficulties should not detract from the public health importance of *Campylobacter* infection across Europe. The World Health Organisation estimates that approximately 1% of the population of Western Europe will be infected with *Campylobacter* each year (Humphrey *et al* 2007).

Campylobacter also causes a considerable economic burden. The average cost of a case of acute *Campylobacter* infection (excluding long-term sequelae) in England in 1995 was estimated to be £1315. Therefore it has been conservatively estimated that food-borne *Campylobacter* infection costs

the UK at least £65 million per annum and the true figure is probably closer to £500 million per annum (Humphrey *et al* 2007).

1.3 Clinical features of *Campylobacter* infection

The incubation period for *Campylobacter* ranges from 1 to 11 days, but is usually 2-5 days. Infection results in an acute self-limiting gastrointestinal illness characterised by diarrhoea, abdominal pain and fever and in some cases also nausea and vomiting lasting for about a week (Acheson & Allos 2001). Results from the participation of Lothian NHS Board in the UK *Campylobacter* Sentinel Surveillance Scheme (2001-2002) showed that despite generally being a self-limiting illness, *Campylobacter* infection leads to hospitalisation of 6.6% of cases (Smith-Palmer & Cowden 2003). This is similar to number observed in England (Gillespie *et al* 2006). Bacteraemia is detected in <1% of patients with *Campylobacter* infection and is most likely to occur in those with a reduced immune function (Skirrow *et al* 1993).

Deaths from *Campylobacter* infection are uncommon and occur primarily in infants, the elderly and patients with underlying conditions. A study in England and Wales estimated that in 2000 there were 86 deaths due to indigenous food-borne *Campylobacter* infection (Adak *et al* 2002). However the authors noted that this figure did not include the approximately 22% of cases who acquire their infection abroad or the 20% of indigenous cases believed not to be food-borne.

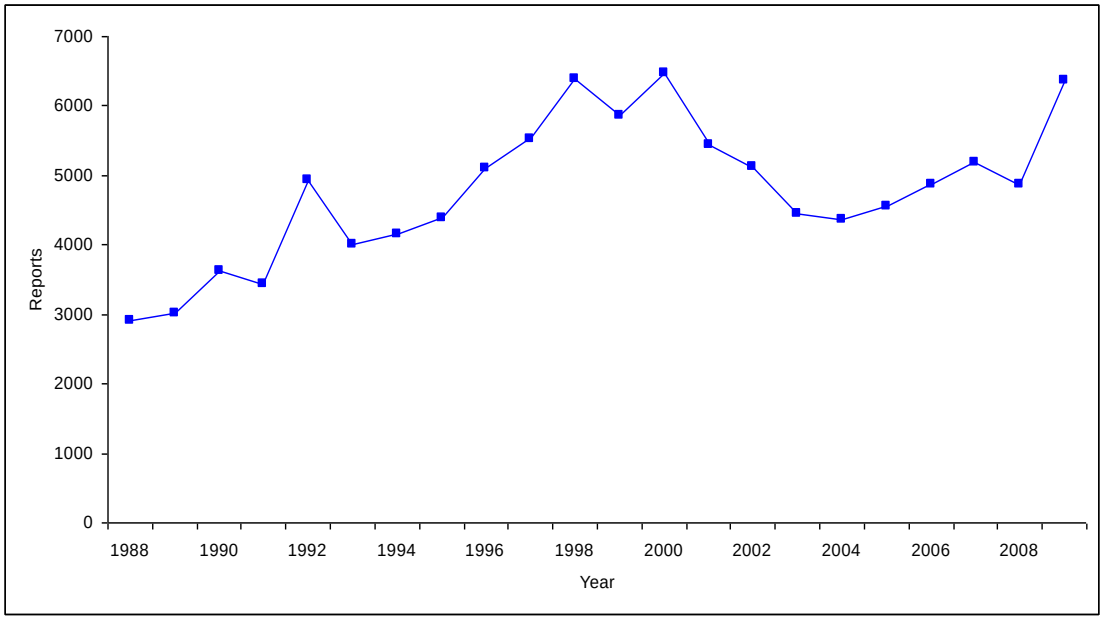
Post infection sequelae, the most important of which is Guillain-Barré syndrome (GBS), a demyelinating disorder resulting in acute neuromuscular paralysis, may also occur following *Campylobacter* infection. The risk of developing GBS after *Campylobacter* infection is about 1 in 1000, and is higher with certain serotypes (Allos 1997). Estimates vary for the percentage of GBS cases that occur after *Campylobacter* infection. One study found recent infection with *Campylobacter* was evident in up to 40% of patients with GBS (Allos 1997), similar to that reported in work of Hughes and Cornblath (2005) in which about a quarter of patients with GBS had a recent *C. jejuni* infection, while another study estimated that *C. jejuni* was responsible for approximately 15% of all cases of GBS in England (Tam *et al* 2003). *Campylobacter* infection has also been associated with the development of the related illness, Miller Fisher syndrome, a localised variant of GBS (Overell & Willison 2005) as well as reactive arthritis (Hannu *et al* 2002).

1.4 Epidemiology of *Campylobacter* in Scotland

In 2009, 6378 isolates of *Campylobacter* from humans were reported to HPS. All laboratory isolates from humans are routinely reported to HPS voluntarily by the clinical microbiology laboratories in Scotland. It is recognised that these laboratory reports represent only a fraction of the true incidence of *Campylobacter* infection, as only a proportion of cases would seek medical attention and only a proportion of these would be requested to submit a stool sample for analysis and of those requested to do so, not all will submit a sample. Therefore it is likely that those with microbiologically confirmed infection represent the more severe end of the clinical presentation of the illness and also particular age groups. A study in England estimated that for

every case reported at a national level another 7.6 go unreported in the community (Wheeler *et al* 1999). There is no reason to suppose that the situation in this respect is notably different in Scotland. However, the study in England collected data between 1993 and 1996, in the intervening years there may have been changes in the epidemiology of *Campylobacter* including an increase in resistant strains or a changing profile of strains responsible, which would be hard to identify due to the lack of routine typing. Alternatively, there may have been a shift in the criteria used or attitudes towards requesting a patient to submit a stool sample. Despite these limitations the study highlights the extent to which laboratory reports represent only a fraction of the true burden of *Campylobacter* infection.

Figure 1: Laboratory reports of *Campylobacter* to HPS 1988 to 2009



During the early 1980s there was a steady rise in reports of *Campylobacter* infection. Some of this initial rise can be attributed at least in part to developments in the laboratory isolation and identification of *Campylobacter.* The rise in the late 1980s and the 1990s cannot be explained by such developments and is believed to be a genuine increase although the reasons for this remain unknown.

Infection in Scotland peaked in 2000 when 6482 isolates were reported and then declined every year to 2004 when there were 4365 isolates. Between 2004 and 2007 reports increased with 5194 isolates reported in 2007 followed by a small decline in 2008 with 4878 reports, this was then followed by a 30% increase in 2009 with 6378 isolates, taking the incidence to only slightly below the peak of 2000, the reasons responsible for this dramatic increase are not yet understood.

A similar trend in the rise and fall in *Campylobacter* has been observed in other countries including England and Wales (HPA 2006). Interestingly, although a similar trend has also been observed in America, the decline began earlier in 1996 (Samuel *et al* 2004). It is unclear as to why the decline

should have started earlier in America. No one factor has been identified as being responsible for this rise and fall. Identifying responsible factors is complicated by the lack of routine typing of isolates to distinguish if some of the trend has been associated with the emergence and decline of particular strains.

1.5 Epidemiology and Multilocus Sequence Typing (MLST) of *Campylobacter* in Scotland

The epidemiology of *Campylobacter* in Scotland has recently been investigation via the use of molecular typing (MLST) in a study conducted by University of Aberdeen. The main goal of the study was to use MLST to provide quantitative attributions of clinical *Campylobacter* infections to infection sources in Scotland.
(www.food.gov.uk/multimedia/pdfs/publication/fullreportcamps.pdf)

MLST categorises each isolate as a sequence type (ST) according to its allele profile across the set of genes. Isolates matching for the whole set of genes are categorised as being the same ST. Isolates mismatching for one gene of the set are defined as single-locus variants (SLV) and are categorised as being in the same clonal complex (CC). Isolates of the same ST or CC are assumed to have a common ancestor, which is believed to be more recent for isolates in the same ST than for isolates in the same CC.

During the study *Campylobacter* isolates from clinical infections were obtained for the period from July 2005 to September 2006. *Campylobacter* isolates were also obtained from host and food sources comprising farm species, wild birds, companion animals and retail chicken and offal.

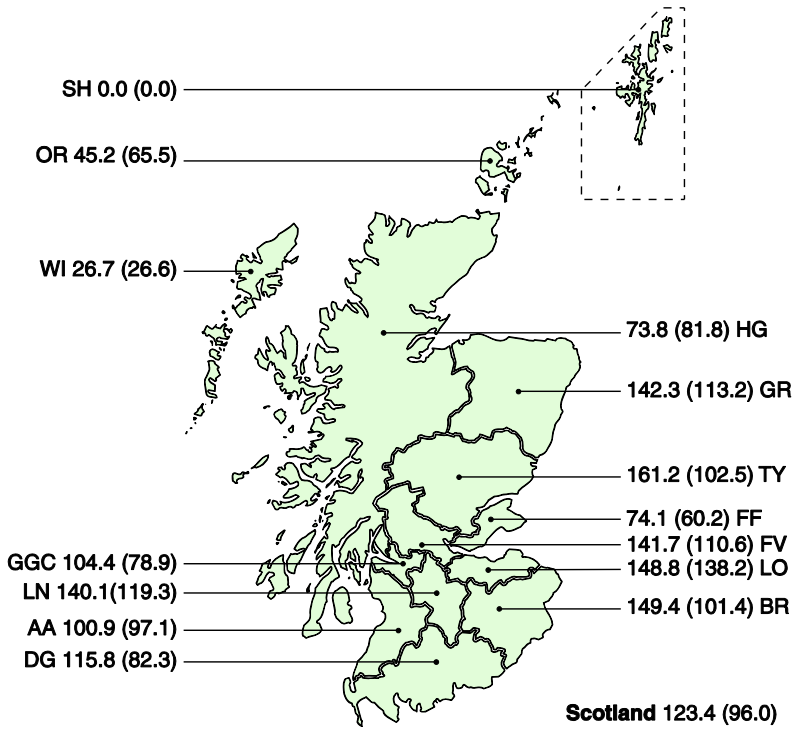
Clinical strain diversity was homogeneous across the 12 mainland NHS Boards, suggesting that clinical infection is homogeneous across Scotland. Clinical strain diversity was slightly heterogeneous across months with certain STs having pronounced peaks of occurrence during spring and summer months.

Approximately three-quarters of clinical isolates could be attributed to each of six potential infection sources: less than 1% to pigs, 5-6% to wild birds, 12-15% each to cattle, sheep and companion animals, and just over 30% to retail chicken. The study reported retail chicken as the single largest source of clinical *Campylobacter* infection in Scotland, consistent with well-known *Campylobacter* prevalence and bacterial loads in broiler chickens and with case-control studies. The study also reported farm ruminants as important sources, with approximately one-third of *Campylobacter* strains infecting humans attributed to farm ruminants. The attribution to farm animals was the most controversial finding of the study, as confirming studies are rare and infection routes are uncertain.

1.6 Variation in rates of infection across Scotland

An interesting feature of *Campylobacter* infection in Scotland is the large variation in reported rates between different NHS boards.

Figure 2: Rates per 100,000 population of reports of *Campylobacter* to HPS, 2009.



The overall rate of reported infection in 2009 was 123.4 per 100,000 population. Rates varied widely between the NHS boards from 0 in Shetland to 161.2 in Tayside. The rates for the Island NHS boards should be viewed with caution due to the effect of their small population size. The rate in Grampian is usually among one of the highest rates in Scotland and for 2009 was 142.3 per 100,000

1.7 Risk factors for *Campylobacter* infection

A range of risk factors have been reported for *Campylobacter* infection. The identification of such factors is usually achieved either via the investigation of outbreaks or epidemiological studies in particular case control studies. It should be taken into consideration that reported outbreaks may not be a true reflection of the epidemiology of *Campylobacter* infection. Firstly, outbreaks account for only a small proportion of all *Campylobacter* cases. Secondly, the lack of routine typing of *Campylobacter* makes it difficult to identify outbreak cases from the background of sporadic cases, and therefore many small outbreaks may remain unrecognised. Thirdly, outbreaks reported in peer reviewed journals may be unusually large or particularly interesting in some aspect compared to unpublished outbreaks and therefore may not be representative of all outbreaks.

A range of risk factors have been associated with *Campylobacter* infection, including travel abroad, contact with animals, food and water consumption. Even in the best of studies the identified factors seldom account for more than 50% of cases.

Travel abroad has been one of the most widely identified risk factors for *Campylobacter* infection reported from a number of studies, including Unicomb *et al* 2008, Neimann *et al* 2003, Rodrigues *et al* 2001, Neal & Slack 1997, Eberhart-Phillips *et al* 1997 and Schorr *et al* 1994.

Campylobacter infection has also been associated with animal contact, however this has proved to be significant in some studies but not in others. Contact with pets was reported by Carrique-Mas *et al* 2005 and Neal & Slack 1997, but not found to be significant by Rodrigues *et al* 2001, while other studies have found an association with contact with cattle (Evans *et al* 2003, Eberhart-Phillips *et al* 1997) or with having an occupational exposure to animals (Kapperud *et al* 2003)

Considerable variation exists amongst the various case control studies in the role of poultry consumption as a risk factor. Some studies have reported eating poultry to be a risk factor (Evans *et al* 2003, Neal & Slack 1997, Schorr *et al* 1994), whereas others only found a risk with consumption of undercooked poultry (Michaud *et al* 2004, Neimann *et al* 2003, Eberhart-Phillips *et al* 1997, Stafford *et al* 2007), poultry bought raw (Kapperud *et al* 2003), or poultry eaten in a restaurant (Unicomb *et al* 2008, Michaud *et al* 2004, Friedman *et al* 2004, Rodrigues *et al* 2001, Eberhart-Phillips *et al* 1997). The study of Rodrigues *et al* (2001) found no significant risk with the consumption of poultry prepared and eaten in the home, only when eaten in a restaurant. In a recent Danish study the main risk factor identified was eating fresh and unfrozen chicken. In contrast other poultry including previously frozen chicken was of borderline significance (Wingstrand *et al* 2006). In the study of Carrique-Mas *et al* (2005) eating poultry was not a significant risk and in some studies had even been shown to be protective, i.e. the consumption of baked or roast chicken was protective in a study by Eberhart-Phillips *et al* (1997), a protective effect was also observed by Adak *et al* (1995). These findings of a possibly protective effect related to poultry consumption leave many unanswered questions.

A number of other foods have also been reported as a risk factor for *Campylobacter* infection, including unpasteurised milk (Neimann *et al 2003,* Eberhart-Phillips *et al* 1997) or milk from bird pecked bottles (Neal & Slack 1997). Food from a barbeque has been also identified (Kapperud *et al* 2003, Neimann *et al* 2003), as has undercooked pork (Kapperud *et al* 2003), undercooked beef and eating at a restaurant (Gallay *et al* 2008), eating poultry liver (Schorr *et al* 1994) and offal (Stafford *et al* 2007) as well as drinking bottled water (Evans *et al* 2003).

Private and untreated water supplies have also been associated with *Campylobacter* infection, in particular in studies conducted in the Nordic countries. A case control study in Norway identified drinking undisinfected

water as a leading risk factor. In this study cases were more likely than controls to use undisinfected water in their household (Kapperud *et al* 2003). A case control study from Finland reported drinking dug-well water to be a risk factor for *Campylobacter* infection. The same study also reported swimming in natural sources of water was a novel risk factor (Schönberg-Norio *et al* 2004). A Swedish study of risk factors for domestically acquired *Campylobacter* infection among children aged less than 6 years, found a risk associated with having a well in the household and drinking water from a lake/river (Carrique-Mas *et al* 2005)

Work from New Zealand by Eberhart-Phillips *et al* (1997) found an association with consumption of certain untreated water sources, including 'non-city water outside the home in 10 days prior to onset' and 'rainwater source for home water supply'. These authors state that although the association with rainwater as a home water source has not been described elsewhere at the time of this publication, it is biologically plausible. These systems are typically untreated and wild birds, which are a major animal reservoir for *Campylobacter* species, can easily contaminate them by roosting on the roof where the rainwater is collected.

There are also a number of reports of general outbreaks due to water contaminated with *Campylobacter,* including an outbreak in 2002 in Sweden associated with a municipal water supply (Martin *et al* 2006). Between 1980 and 2003 there were 20 waterborne outbreaks of *Campylobacter* reported in Sweden, involving 11,608 cases. The three largest occurred in 1980, 1994 and 1995 with respectively, 1000, 2500 and 3000 affected (Stanwell-Smith *et al* 2003). In an outbreak in northern Finland drinking non-chlorinated municipal tap water was strongly associated with illness (Kuusi *et al* 2005). Waterborne outbreaks associated with contamination of drinking water by *C. jejuni* are rather common in the Nordic countries Sweden, Norway and Finland, where in sparsely populated districts groundwater is commonly used without disinfection (Moore *et al* 2005). An outbreak in a small rural community in Canada was associated with a potable water supply which was unfiltered and not chlorinated (Alary & Nadeau 1990). Investigation of an outbreak on a resort island in north Queensland indicated that untreated rainwater was the most likely source. The authors postulated that the droppings of wild animals carrying *Campylobacter* contaminated one or more of the rainwater tanks with *Campylobacter* (Merritt *et al* 1999). *Campylobacter* was one of the pathogens identified in a large waterborne outbreak of multiple aetiologies on South Bass Island, Ohio, in which sewage-contaminated ground water was the likely source (O'Reilly *et al* 2007).

A review of outbreaks of infectious intestinal disease associated with private water supplies in England and Wales 1970-2000, found that *Campylobacter* was the main pathogen, implicated in 52% of outbreaks. Most outbreaks (88%) occurred in commercial or Category Two supplies, which potentially affect larger populations. The main factors implicated in these outbreaks were temporary or transient populations, lack or failure of treatment, the presence of animals and heavy rains (Said *et al* 2003). In Scotland, ObSurv is the surveillance system established in 1996 for surveillance of all general

outbreaks of infectious intestinal disease. For the purpose of ObSurv an outbreak is defined as an incident in which two or more linked cases experience the same illness or when the observed number of cases unaccountably exceeds the expected number. The system seeks information on general outbreaks defined as outbreaks affecting members of more than one household or residents of an institution. Between 1996 and 2007, 28 general outbreaks of *Campylobacter* were reported to ObSurv. In six (25%) of these the main mode of transmission was described as mainly waterborne. It is recognised that ObSurv covers only general outbreaks, and therefore would not have included any outbreaks associated with private water supplies that were restricted to just a single household. Even outbreaks affecting only a couple of households may be hard to identify, especially where not all of those affected seek medical attention and submit a stool sample.

Little is known about the role of private water supplies in sporadic cases of *Campylobacter* infection, especially in Scotland, where no previous research has been undertaken to investigate this particular factor. Analysis of data from the Scottish component of a UK-wide *Campylobacter* Sentinel Surveillance Scheme (2001-2003) found that 8% of *Campylobacter* cases taking part in the study from Lothian NHS board area reported the consumption of cold unboiled water from a private supply in the two weeks before the onset of illness. However, the Drinking Water Quality in Scotland 2002 report states that a much lower proportion of the population in NHS Lothian have a private water supply (0.44% for East Lothian, 0.03% for Edinburgh City, 0.54% for Midlothian and 0.15% for West Lothian) suggesting that private water supplies could be a potential cause of human *Campylobacter* infection. As this study was a sentinel study it was able to suggest factors for further investigation, rather than being able to quantify the risk as it is possible with cohort or case control studies.

1.8 Private Water Supplies in Scotland

It is estimated that around 150,000 people in Scotland rely on a private water supply for their drinking water. Tens of thousands of people also use them each year, typically when they are on holiday. The quality of water from private supplies is highly variable and when poor can cause significant health problems (Drinking Water Quality in Scotland 2005).

A private water supply is any supply which is not provided by the statutory water undertaker, which in Scotland is Scottish Water. Private water supplies are classified as Type A or Type B. Under the Private Water Supplies (Scotland) Regulations, 2006, types of supply are defined as:

Type A – Supplies providing $10m^3$ of water a day or serving 50 or more persons; and supplies to commercial or public activities irrespective of their size.
Or
Type B – Supplies serving only domestic premises with less than 50 persons supplied.

Type A supplies attract mandatory monitoring and enforcement of water quality standards by local authorities, whereas Type B supplies are subject to a discretionary regime.

The Private Water Supplies (Scotland) Regulations 2006 came into force on 3rd July 2006 and replaced The Private Water Supplies (Scotland) Regulations 1992. While the primary driver for legislative change was the revised Drinking Water Directive from the European Commission (Council Directive 98/83/EC, November 1988), other drivers included the World Health Organisation Guidance on Drinking Water Quality (3rd Edition) and the Scottish Executive *E. coli* O157 Task Force Report of June 2001. The overriding objective of the new regulations was to ensure the provision of clean and wholesome water to rural communities and rural businesses in Scotland. It should be noted that the primary legislation pertaining to water supplies in Scotland, including private water supplies, remains the 1980 Act (Drinking Water Quality in Scotland 2005).

The new approach to regulation has been to shift the whole regulatory effort away from 'end of pipe testing', i.e. testing the water that emerges from a tap, towards a more pro-active approach based around risk assessment, i.e. trying to identify potential problems before they occur and taking appropriate steps to reduce or eliminate the risks such problems pose. The Private Water Supplies (Scotland) Regulations 2006 adopt this new approach by incorporating risk assessment as part of the core philosophy underpinning the Regulations. The new regulations require local authorities to find out the cause of a supply failure and initiate remedial action. Risk assessments are an essential element of effective drinking water quality surveillance and control. Local authorities are under a duty to complete a risk assessment for the Type A supplies and to provide information and support to enable owners to complete a risk assessment for the Type B supplies (Drinking Water Quality in Scotland 2005).

CHAPTER 2: AIMS

The aim of the study was to investigate the role of private water supplies as a risk factor for *Campylobacter* infection in Scotland.

Private water supplies have previously been identified as a risk factor for *Campylobacter* infection as a result of the *Campylobacter* Sentinel Surveillance Study conducted in NHS Lothian. Private water supplies have also been associated with outbreaks of *Campylobacter* and other enteric pathogens, but no work has been conducted to establish their importance in sporadic cases.

The study was conducted in Grampian NHS board for three principal reasons. Firstly, the rate per 100,000 for *Campylobacter* infection in Grampian is consistently one of the highest for NHS boards in Scotland. Secondly, 13.1% of the population of Aberdeenshire have a private water supply, the highest proportion of any local authority area in Scotland and thirdly NHS Grampian Health Protection Team was willing to be involved in the study.

2.1 Primary Aim

To identify whether the consumption of water from private water supplies is a risk factor for *Campylobacter* infection in Aberdeenshire and Aberdeen City

2.2 Secondary Aims

To identify whether

- Private water supplies play a role in the seasonality of *Campylobacter* infection
- There is an association between particular molecular (MLST) types of *Campylobacter* and private water supplies.

CHAPTER 3: METHODS

3.1 Overview of study methodology

The study was a case control study that investigated private water supplies as a risk factor for *Campylobacter* infection. One of the aims of the study was to investigate if private water supplies play a role in the seasonality of human *Campylobacter* infection. In order to achieve this it was necessary for data collection to be carried out over a two year period (August 2005-November 2007). The study collected epidemiological data and a sample of tap water from the home supply for microbiological testing from cases of *Campylobacter* infection and frequency matched controls in Aberdeenshire and Aberdeen City over a period of 24 months. Water samples were only collected from mains supplies for the first 12 months of the study.

The case control study coincided with another Food Standards Agency in Scotland funded project (FSAS project S14006) which analysed and compared MLST profiles for all clinical *Campylobacter* isolates in Scotland, thereby also allowing the incorporation of MLST data into this study and the investigation of any association between particular MLST profiles and private water supplies.

3.2 Ethics Approval

Approval for the study was obtained from Grampian Research Ethics Committee. Amendments were submitted to the ethics committee as appropriate (Appendix B).

Approval for the study was also obtained from Grampian NHS Research and Development committee (Appendix B).

3.3 Sample size

In Aberdeen City and Aberdeenshire 6% of the total population relies on a private water supply (13.1% of the population of Aberdeenshire and 0.15% in Aberdeen City (Drinking Water Quality in Scotland 2002)) Assuming a 6% exposure to private water supplies in the region covered by this study and allowing two controls for every case of *Campylobacter* infection, with a sample size of 400 cases (and 800 controls) the power to detect an odds ratio of 2 is 0.88. As approximately 50% of cases were expected to occur in Aberdeenshire, it was also possible to perform the analysis on Aberdeenshire only. Assuming that in Aberdeenshire 13% of the population have private water supplies, for 200 cases with two controls per case, the power is 0.86 to detect an odds ratio of 2.

3.4 Cases

A case was any person belonging to the population of Aberdeenshire or Aberdeen City registered with a GP in Grampian NHS Board who had culture confirmed *Campylobacter* infection identified by the microbiology laboratory at Aberdeen Royal Infirmary which was reported to NHS Grampian Health Protection Team. The testing protocol for faecal samples was the standard protocol used by Aberdeen Royal Infirmary and tests not only for *Campylobacter* but also for a range of other gastrointestinal pathogens.

A study information pack was sent to all cases by the Health Protection Team inviting them to participate in the study. The pack contained an invitation letter, leaflet about *Campylobacter* infection, study information sheet, consent form, decline form, case questionnaire, and pre-paid reply envelope addressed to HPS (Appendix C).

The information sheet for cases explained that there were two parts to the study: a questionnaire and microbiological testing of a water sample from their home supply. It was explained that they were free to participate in just the questionnaire component, both the questionnaire and water testing component or decline participation. If they wished not to take part in the study they were asked to return the decline form in the envelope provided. The information sheet was amended in the second year of the study to reflect the change in the water testing component to only test those on a private water supply.

The case questionnaire collected basic demographic information, date of onset and clinical presentation of infection, details of others in the household with similar symptoms, details of any overnight stay outside Aberdeenshire or Aberdeen City in the 14 days prior to onset, food and water consumption, animal contact and recreational water activities in the five days prior to onset, type of household water supply (mains or private water supply) and if a private water supply the date it was last tested if known.

For cases that agreed to participate in the water testing component of the study, their name and contact details were passed electronically in a password protected file to the water testing team at the Microbiology Laboratory, University of Aberdeen. The water testing team from the University contacted the case directly and arranged a mutually agreeable time to visit and collect the water samples.

For cases that were younger than 16 years the case information pack was sent to the parent/guardian inviting them to participate on the child's behalf. The case information sheet to the parent/guardian was accompanied by a simplified version of the case information sheet designed for young people.

A reminder letter was sent where no reply, either decline form or consent form and questionnaire was received from cases within 14 days. If no reply was received in response to the reminder no further contact was made with the case. A 'thank-you' letter was sent to cases on receipt of the completed questionnaire.

All case questionnaires that reported that the case had been admitted to hospital or was aware of others with similar symptoms or contained other relevant public health information were converted to a PDF file and emailed to the Health Protection Team for any appropriate public health action.

3.5 Controls

The study aimed to recruit two controls for each case, in order to achieve this five controls were contacted for each case.

Controls were selected randomly from the Community Health Index (CHI) for Grampian; this is a register of all individuals registered with a General Practice in Grampian, controls were only selected if resident in Aberdeenshire or Aberdeen City. Controls were frequency matched based on the number of cases by sex and aged band reported in the previous two years to estimate number and distribution of cases expected each month. The selection of controls was undertaken by the data manager at the College of Life Sciences and Medicine, University of Aberdeen, who had an honorary contract with NHS Grampian. A list of cases was provided by the Health Protection Team on a weekly basis and these individuals were excluded from the list of potential controls invited to participate, such that a case could not later be asked to participate as a control.

Controls were selected and control study packs sent once a month. The information sheet for controls was similar to that for cases and explained that they had been selected at random to participate in the study. The control questionnaire was similar to that for cases but asked about exposure history in the previous five or 14 days as appropriate, rather than the five or 14 days before onset of illness.

As with cases, controls were invited to participate in the water testing component of the study and the procedure was the same as for cases. The water testing team were not informed by HPS whether a participant was a case or control.

The same procedure as for cases was followed for controls where no reply was received from controls within 14 days.

All control questionnaires that reported that the control had suffered from bloody diarrhoea were converted to a PDF file and emailed to the Health Protection Team.

During the analysis stage, the analysis was conducted using all controls and also with only ‘well’ controls. Well controls were those who reported no symptoms of diarrhoea, bloody diarrhoea, vomiting or abdominal pain, in the five days prior to completing the questionnaire. The exclusion during this part of the analysis of controls who reported any of the symptoms compatible with *Campylobacter* infection, removed any controls that could have potentially been undiagnosed cases.

3.6 Water testing component of study

The name and contact details of participants consenting to take part in the water testing component of the study were provided to the water testing team at University of Aberdeen in a password protected file, on a daily basis. The water testing team from the University contacted the participant directly and arranged a mutually agreeable time to visit and collect 2 X 5 litre water

samples. The taps were disinfected prior to the collection the of the water samples. The 5 litre bottles used for collection of the water samples contained sodium thiosulphate to neutralise the activity of any residual chlorine.

Samples were tested for *Campylobacter,* Enterococci*,* Coliforms, *E. coli* and *E. coli* O157. For details of the microbiological testing methods see Appendix D.

The water testing results, accompanied by the appropriate advice, were reported to the participant by University of Aberdeen. During the first year of the study if the supply failed to meet the requirements of the Private Water (Scotland) Supply regulations 1992, when 9 or less coliforms were detected per 100 ml and no *E. coli* were detected and the results letter was sent to the participant. It informed them that although the sample failed to meet the prescribed standard in that coliform bacteria were present, no faecal coliforms were detected and the supply did not appear to present an imminent risk to health. The participants were advised that they should consider having the entire water supply cleaned and disinfected, and that an inspection of the existing supply should be carried out for any signs of disrepair or contamination and they may wish to consider the installation of an appropriate filter to safeguard the supply in the long term.

Where water supplies failed to meet the requirements of the Private Water (Scotland) Supply regulations 1992 by having more than 9 coliforms per 100 ml detected, the participant was advised that the entire water supply should be cleaned and disinfected. Until that has been undertaken and satisfactory water samples obtained they were advised that the water supply should not be used for drinking or culinary purpose without first being boiled. Furthermore, it was suggested that an inspection of the existing water supply should be carried out for any signs of disrepair or contamination and that they may wish to consider the installation of an appropriate filter to safeguard the supply in the long term.

As a result of the changes in private water regulations in 2006, in the second year of the study, all participants where there was any failure of the water supply were advised that the water supply should not be used for drinking or culinary purpose without first being boiled.

All results were copied to NHS Grampian Health Protection Team, Aberdeenshire Environmental Health and HPS. Results of any failures were reported to NHS Grampian Health Protection Team and Aberdeenshire Environmental Health, as soon as these were detected by the laboratory whilst all other results were reported on a weekly basis.

Any *Campylobacter* isolated through the water testing were sent for MLST typing as part of the MLST study.

3.7 Amendment to study during second year of the study

As a consequence of the water testing results obtained during the first year of the study, the study was amended such that only those on private water

supplies were asked to participate in the water testing component of the study.

An amendment was submitted to Grampian Ethics committee to cover this change to the study protocol and the study information sheets to cases and controls updated accordingly.

3.8 MLST study (FSAS project S14006)

The collection of clinical samples carried out as part of the MLST project was extended in Grampian to cover the whole duration of the *Campylobacter* case control study.

The MLST data was linked to the epidemiological data collected from cases. Linkage was based on case's date of birth, where more than one isolate with the same date of birth was included, the date of onset of the illness and date when the isolate was received for typing were considered, and where available also the postcode.

3.9 Details of private water supplies

Data files of all known private water supplies were provided by Aberdeenshire and Aberdeen City Environmental Health Departments. This allowed the information provided in the questionnaires to be cross-checked and information on the type of supply (type A or B) added to the database.

The sources of water supply provided in the study questionnaire were validated using the lists of private water supplies provided by Aberdeenshire and Aberdeen City Environmental Health Departments as well as the observations made by the water testing team when visiting the house and collecting the water sample (for those who participated in the water testing component of the study). As a result of these observations a derived field was created with the final water source assigned to each participant to be used in the analysis.

3.10 Other data sources used

Data on the age structure of the population of Aberdeen City and Aberdeenshire was obtained from the annual population data published by the Scottish Government (www.gro-scotland.gov.uk/statistics/publications-and-data/population-estimates).

Data on deprivation category was obtained from the Carstairs report based on the information from the 2001 census data. The scores had been calculated from a combination of four variables derived from the census data. The four variables were no car ownership, male unemployment, overcrowding and social class IV & V.

Scottish Index of Multiple Deprivation (SIMD) was obtained from the Scottish Government (http://www.scotland.gov.uk/Resource/Doc/47251/0027011.pdf). The index brings together 31 different indicators which cover specific aspects of deprivation: current income, employment, health, education, housing and access. These are combined to create the overall SIMD. The SIMD is

converted to rank from the most deprived to the least deprived area. The lower the SIMD score the higher the SIMD rank and the less deprived the area. For example an area with an SIMD score of 3 has a rank of 6312, while an area with a score of 39 has a rank of 902. The SIMD is not a measure of affluence. The indicators which were used in the SIMD were chosen for their representative of deprivation and a lack of deprivation does not necessarily equate to affluence. Therefore data zones with the highest ranks are not necessarily affluent, just less deprived.

The coding of occupation groups was based on Standard Occupational Classification 2000 Volume 1 – June 2000.

Missing postcodes were accessed from Royal Mail Postal Address Book, Scotland 1 & 2 (2001) and Quick Address Pro application

Data on the total number of laboratory confirmed cases of *Campylobacter* infection reported from NHS Grampian, was that available at HPS as part of the voluntary routine reporting of all cases by all NHS laboratories in Scotland.

3.11 Data storage

Data from the case and control questionnaires and results from the water testing were entered onto a password protected database at HPS. Access to the folder containing the database was restricted to the epidemiologist and study administrator responsible for the study. All paper questionnaires were stored at HPS in locked cabinets.

3.12 Pilot study and study dates

A pilot study was conducted prior to the start of the main study. The pilot study was conducted for two months in August and September 2005. Questionnaires continued to be sent to cases during October. Those received by HPS up to 31st October 2005 were included in the pilot study and those received from 1st November 2005 onwards were contained in the main study. Data collection for the main study was conducted from November 2005 to November 2007. Questionnaires received at HPS until 7th January 2008 were included in the study database.

3.13 De-duplication and cleaning of the database

The database was interrogated for duplicates, defined as cases with the same name, date of birth and onset date, and the duplicate record removed. Duplicates of cases and controls could arise as a small number of participants returned the reminder questionnaire, which was sent before the original completed questionnaire was received at HPS. The second of the questionnaires received was removed, with any additional information in the second questionnaire which was not present in the original added to the database entry for the original questionnaire.

Cases that were part of recognised general outbreaks were removed from the database before the analysis was conducted. The definition of an outbreak applied was that used by ObSurv: the surveillance system for all general outbreaks of infectious intestinal disease in Scotland, an outbreak is defined

as an incident in which two or more linked cases experience the same illness or when the observed number of cases unaccountably exceeds the expected number. The system seeks information on general outbreaks, defined as outbreaks affecting members of more than one household or residents of an institution.

Cases and controls that were not resident in Aberdeenshire or Aberdeen City were also removed from the database. A few participants were resident outside the study area. Their invitation to participate in the study arose either by an error in the selection of control/cases or because the participant visited the area at the time the sample was submitted and therefore had a temporary address within the study area, but was not a permanent resident of the study area.

The database was cleaned, to remove any data entry errors that may have occurred, by running a number of frequencies and cross-tables, for example Adult/Child status against age band, occupation group. Where any discrepancies were found these were investigated by looking at the original paper form and the data re-entered if necessary. As some data entry errors were detected, all paper questionnaires were then checked against the data in the database and any data entry errors corrected. A record log was maintained for all changes made to the database and reason for the change.

3.14 Data analysis

3.14.1 odds ratios, adjusted odds ratios

Data analysis was conducted using Excel, SPSS and EpiInfo 6.

The comparison of risk related to different factors between cases and controls was conducted by determining simple odds ratios.

Potential confounding effects of known risk factors for *Campylobacter* were assessed through fitting logistic regression models adjusting for both age and sex. For each of the major factors identified in the study, e.g. travel etc, a logistic model was fitted including the effects of both private water supply and the factor. We then added in the interaction between private water supply and the factor and tested the significance of the interaction through a chi square test on the change in deviance. If there is evidence of a significant interaction then this suggests that the effect of private water supply on the odds of having *Campylobacter* is modified by the other major risk factor.

The determination of odds ratios and adjusted odds ratio was conducted using cases and well controls, cases and all controls and then repeated using cases and controls only resident in Aberdeenshire.

When considering the influence of seasonality, the definitions applied to the data were those of the Met Office e.g December, January and February are winter.

3.14.2 Associations between exposure factors, symptoms and hospitalisation

The data for 10 exposure factors, symptoms and hospitalisation were used. All responses other than “yes” “no” were treated as missing data. Associations between all pairs of factors were evaluated using the Cramer’s V statistic (range 0-1, where 0 indicates no association and 1 indicates complete association) calculated using SPSS.

3.14.3 Relationship between Clonal Complexes and categorical variables using Pearson Chi-square and Likelihood ratio chi-square

For this analysis, due to the large number of clonal complexes, many with small numbers of isolates, the analysis was conducted using CC ST 206, ST 21, ST 257, ST 45, ST 48, ST 828 and the remaining CC grouped into other (Appendix Table 62). Associations between these clonal complexes and month of onset, travel outside the study area, travel abroad, eating out, contact with farm animals, contact with pets, chicken eaten outside the home, eating chicken prepared at home, eating red meat prepared at home, having a private water supply and hospitalisation were investigated using Pearson chi-square and likelihood ratio chi-square.

3.14.4 Differences between exposed *versus* unexposed cases in the composition and host attribution of their *Campylobacter* strains.

Exposed *versus* unexposed cases were evaluated for differences in the strain composition and average source attribution of their isolates. Strain composition was quantified at sequence type (ST) level and at the more inclusive clonal complex (CC), which also contained STs of strains with no CC membership.

Strain composition was analysed at two levels. First all strains were combined in two groups (exposed *versus* unexposed) and evidence for an overall difference between the groups was evaluated using an exact test implemented by Markov-chain resampling using the PC package ARLEQUIN (Excoffier *et al* 2005, ARLEQUIN website). Second, each single strain was evaluated for evidence of a difference in occurrence between the two groups using a Fisher’s exact test. Only groups that showed evidence of an overall difference in strain composition were subjected to single-strain analysis. The Fisher’s exact tests were 2-tailed tests and were implemented using an add-in for Microsoft Excel (Fisher’s Exact Test Excel add-in website). The *P*-values were checked using an online calculator (Fisher’s Exact Test online calculator). The 95% confidence intervals of odds ratios were calculated using the standard asymptotic formula for large cell counts.

The false discovery rate framework (Benjamini & Hochberg 1995) was used to evaluate *P*-values from multiple statistical tests for statistical significance and this was implemented using the PC package Q-VALUE (Q-VALUE website).

Cases were also compared according to the average values of attribution of their isolates to four source types: farm ruminants, chicken, pig and wild birds. The attribution values were previously generated from a STRUCTRE analysis (Sheppard *et al* 2009) of strains from known hosts. Differences in average

attribution values were calculated in Microsoft Excel 2003. The observed differences were evaluated for significance by comparison with distributions of 1000 average differences in attribution values generated by random shuffling of the STs. The randomisation was done using the Poptools add-in for Microsoft Excel 2003 (Poptools website).

Strain diversity was quantified as the Hunter-Gaston diversity index (Hunter & Gaston 1988) using the online facility V-DICE (V-DICE website).

CHAPTER 4: EPIDEMIOLOGY RESULTS

4.1 Participation in the study

4.1.1 Total number of questionnaires returned to HPS

Questionnaires were removed from the study if the case/control was resident outside the study area, if they were part of a recognised outbreak or a duplicate. A duplicate was defined as a questionnaire with the same name, address and date of onset or date of completion within a four week period for a control.

Four case questionnaires were removed as duplicates. One of these cases had completed a questionnaire for both her main residence address and holiday home address, both within the study area, the questionnaire relating to the main residence was retained in the study database and the other removed. Six control questionnaires were removed as duplicates.

If a case was laboratory confirmed with *Campylobacter* infection on separate occasions, at least four-weeks apart and participated in the study on each occasion, both episodes of infection were included. This applied to four cases that were each included on two separate occasions.

Five cases and seven controls were removed from the database as they lived outside the study area of Aberdeenshire and Aberdeen City.

Ten outbreak cases were removed from database. These included seven cases from an outbreak associated with a dinner-dance in which pate was the suspected vehicle of infection and three cases from an outbreak associated with a restaurant.

Table 1: Total questionnaires returned and questionnaires included in study

Study	Total questionnaires returned	Questionnaires removed	Questionnaires included
Pilot	182	0	182
Main	2537	32	2505
Total	**2719**	**32**	**2687**

Table 2: Questionnaires included for analysis.

	Cases	Control	Total
Pilot	92	90	182
Main	697	1808	2505
Total	**789**	**1898**	**2687**

4.1.2 Cases and controls who declined to participate

Potential participants had the opportunity to decline participation in the study, by completing and returning the decline form included in the study pack. Sixteen potential participants declined by phoning HPS rather than by returning the decline forms.

In the pilot and main study combined, 128 cases and 646 controls returned decline forms, the remainder of non-participants returned neither a questionnaire or decline form.

4.1.3 Undeliverable questionnaires

Controls were selected at random from the CHI. In some instances the control packs were returned to HPS as the addressee was reported to have gone away or was no longer resident at the address. In total 285 control packs were returned as undeliverable: 11 during the pilot study and 274 during the main study.

4.1.4 Participation rates among cases

NHS Grampian Health Protection Team provided HPS with monthly figures for the total number of laboratory confirmed cases of *Campylobacter* infection resident in the study area, all of which would have been invited to participate in the study. These figures were used to determine case participation rate.

The overall participation rate for cases during the whole study was 59.7% (60.1% during the main study and 56.4% during the pilot).

For cases participation in the study was based on the month the questionnaire was received at HPS, which was not necessarily the same month as date of onset or date the case was notified to NHS Grampian Health Protection Team (Appendix Table 1)

4.1.5 Participation rates among controls

For the selection of controls matching criteria were sent to University of Aberdeen on a monthly basis. The selection criteria were based on the expected number of cases by sex and five year age band.

A total of 5408 controls were invited to participate in the study. It is known that the packs were undeliverable to 285 controls. Assuming that the remaining packs were successfully delivered control packs were received by 5123 potential controls. A total of 1898 controls participated, giving an overall participation rate for controls of 37.0%.

Participation in the study was based on the month in which the questionnaire was received at HPS, which may not necessarily have been the same month as the control was invited to participate in the study. The pilot study was conducted in August and September 2005 and the main study commenced in November 2005, therefore no control packs were sent out in October 2005, however 19 control questionnaires were returned in October as a result of packs being posted to participants in the preceding two months. The lowest participation rates for controls were observed in August 2005 (18.6%) and

November 2005 (16%) but in neither of the preceding months were control packs sent out (Appendix Table 2).

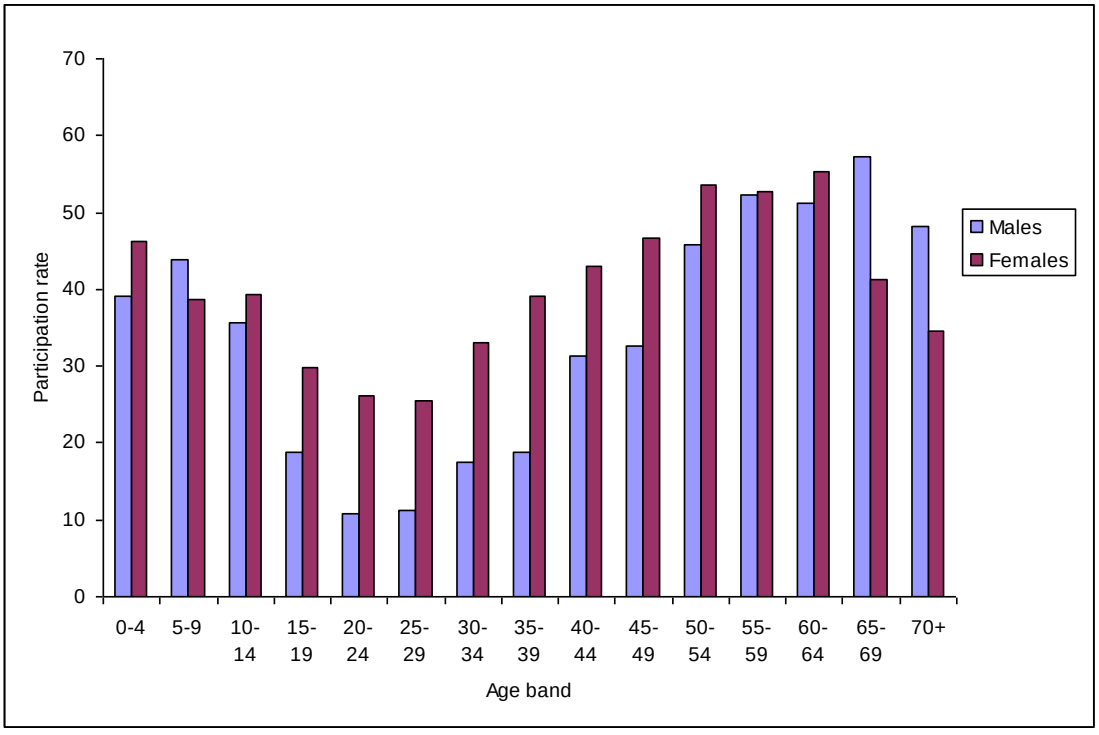
As the selection criteria for controls were based on sex and age group, it was possible to establish the participation rate for the different age groups by sex.

The participation rates presented in Figure 3 are based on controls invited to participate (HPS does not have information on the age of potential controls to whom packs were undelivered). Figure 3 is restricted to the main study only. After reviewing the results of the pilot study, additional control packs were sent to age groups with low participation rates.

The overall participation rate for male controls was 29.2%. Rates ranged from 10.7% for males aged 20-24 years to 57.3% for those aged 65-69 years. In all five age groups for those aged 15 to 39 years the participation rate was below 20%, while for all other age groups it was above 30% (Appendix Table 3).

The overall participation rate for female controls was 38.7%. For female controls, rates ranged from 25.5% for those aged 25-29 years to 55.4% for those aged 60-64 years. Unlike for male controls there were no age bands in which the participation rate was below 20% (Appendix Table 4).

Figure 3: Participation rates among controls in the main study by sex and age band



4.2 Characteristics of cases and controls

4.2.1 Age distribution of cases and controls.

Amongst cases, 13.3% were classified as children, similar to the proportion of children amongst controls 14.3% (Table 3).

The study aimed to achieve a matching frequency of two controls for every case. During the pilot study, a low participation rate was identified among some age groups, in particular young males. As a result the numbers invited to participate in the study was increased for the main study, especially in those age groups that had low matching frequencies.

For females under 1 year, it was not possible to determine a matching frequency as there were no cases (Appendix Table 5)

Among the total of 36 age and gender groups a matching frequency of at least 2:1 was achieved for 72% (27) of the groups (Figure 4).

In the remaining 9 groups a matching frequency was 1:1 or above and in 5 out of these 9 the value was above 1.5:1. Males accounted for 6 (66%) of the 9 groups which did not achieve a matching frequency of 2:1.

The overall matching frequency for the whole study was 2.40:1 (1898 controls and 789 cases), which meant the required frequency of 2:1 was achieved.

There was no significant difference between the age of male cases and controls (p = 0.551), 41.6 and 42.4 years respectively.

There was no significant difference between the age of female cases and controls (p = 0.598), 40.8 and 40.1 years respectively.

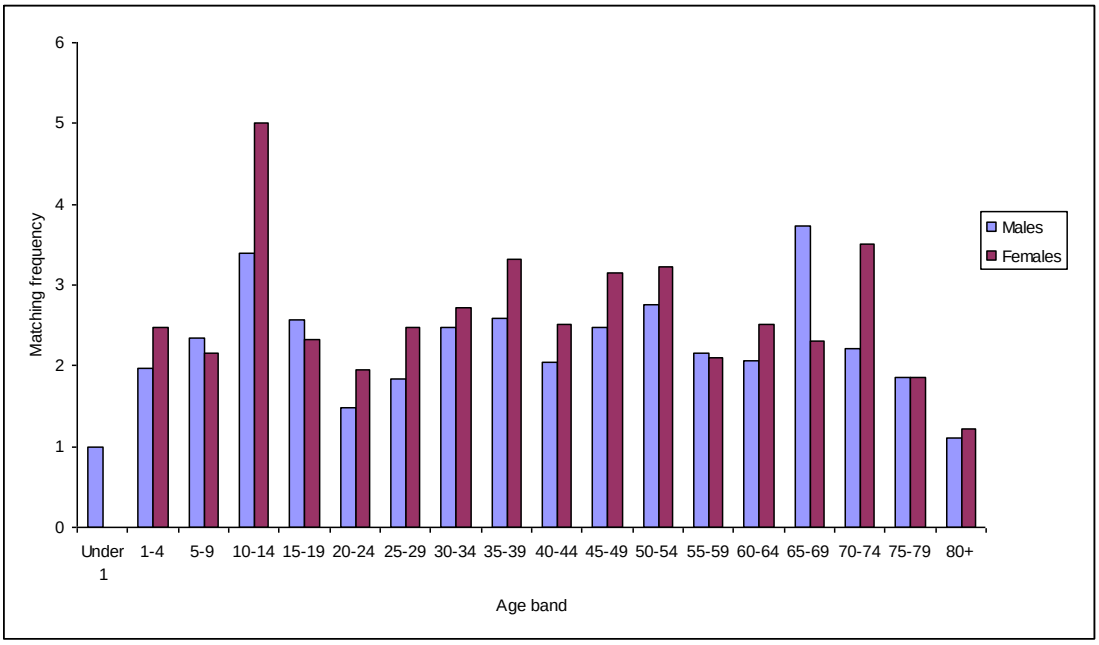
For cases, there was no significant difference (p = 0.565) in the age of males (41.6 years) and females (40.8 years).

For controls, there was a statistically significant difference (p = 0.016) in the age of males (42.4 years) and females (40.1 years). This difference was not believed to alter the overall results or analysis of the study, and probably reflects the difficulties in recruiting young male controls with uptake rates of only 10.7% and 11.2% for the 20-24 and 25-29 year groups compared to 26.1% and 25.5% respectively for females (Appendix tables 3 & 4).

Table 3: Number of adults and children participating in the study.

Study	Cases		Controls	
	Adults	Children	Adults	Children
Pilot	82	10	72	18
Main	602	95	1553	255
Total	**684**	**105**	**1625**	**273**

Figure 4: Matching frequency for males and females



4.2.2 Rates per 100,000 for cases participating in the study and total cases reported to NHS Grampian.

The area covered by NHS Grampian includes Aberdeen City, Aberdeenshire and Moray. The data for all *Campylobacter* cases was that routinely reported to HPS by the microbiology laboratory in Grampian as part of the voluntary reporting for all laboratory reports to HPS.

These rates were calculated for 2006, as this was the only complete calendar year during the study.

Rates per 100,000 by five year age band and sex for cases participating in the study were calculated using as the dominator the population of Aberdeen City and Aberdeenshire only using the Mid-2006 population estimates for Scotland (General Register Office). The rate for all *Campylobacter* cases reported from NHS Grampian used the population of NHS Grampian as the dominator data.

In NHS Grampian, the overall rate of *Campylobacter* infection among males was 122.3 per 100,000. The highest rates were observed in males aged 0-4 years and 20-24 years at 187.0 and 186.9 per 100,000 respectively, the lowest rate of 54.7 per 100,000 was in males aged 10-14 years.

For all male cases participating in the study, the overall rate was 83.2 per 100,000. The highest rate was 145.1 per 100,000 in the 0-4 years group and the lowest rate of 36.9 per 100,000 was in the 10-14 years group. The same groups which had the highest and lowest rates of all cases reported to NHS Grampian. Among the 20-24 years group the rate among those participating in the study was 89.2 per 100,000 less than half that for all cases reported to NHS Grampian of 186.9 per 100,000 (Figure 5).

In NHS Grampian, the overall rate of *Campylobacter* infection among females was 104.4 per 100,000. The highest rate observed in females was 173.3 per 100,000 in those aged 60-64 years, the lowest rate of 25.6 per 100,000 was in females aged 10-14 years.

For female cases participating in the study, the overall rate was 77.1 per 100,000. The highest rate was 136.8 per 100,000 in the 55-59 years group and the lowest rate of 15.6 per 100,000 was in the 10-14 years group. The highest rate among those participating in the study was in a different age group to that for all confirmed cases (Figure 6). The lowest rate was in the same age group in both the study cases and all laboratory confirmed cases. (In the 55-59 age group the rate was higher among cases participating in the study than all laboratory confirmed cases, 136.8 and 113.2 per 100,000 respectively. During 2006 in this group there were 21 laboratory confirmed cases and 21 cases participated in the study. It appears that all these cases were resident within the study area and participated in the study and that none were resident in Moray).

Figure 5: Rates per 100,000 for male *Campylobacter* cases in Grampian NHS Board area and cases participating in the study

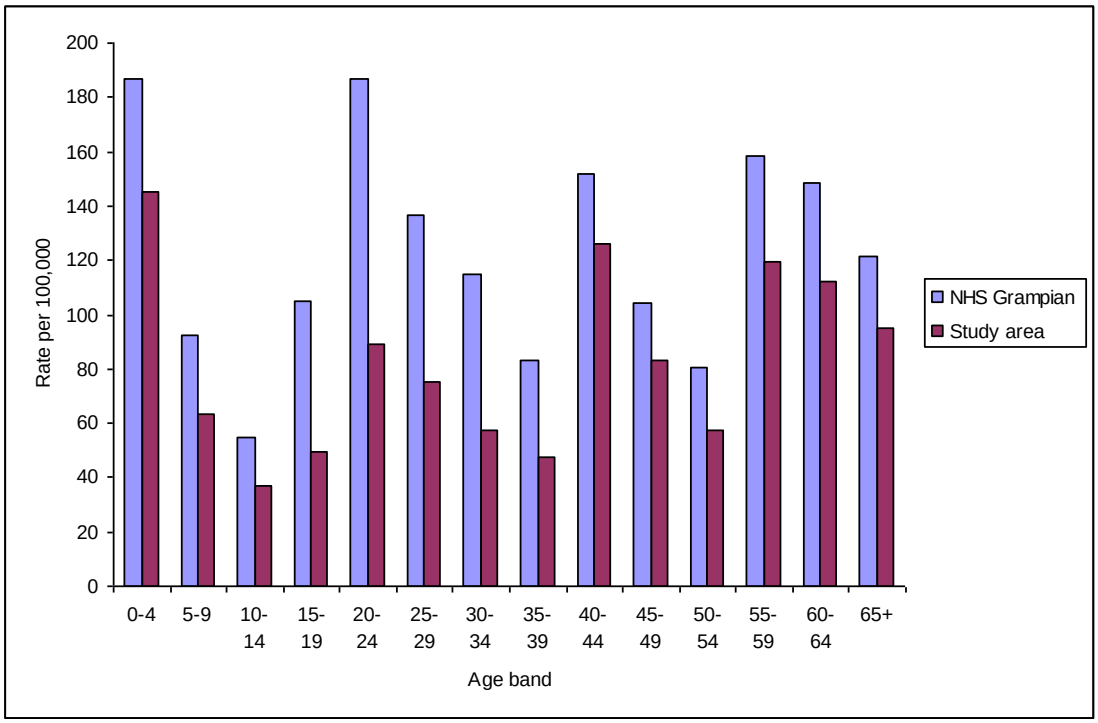
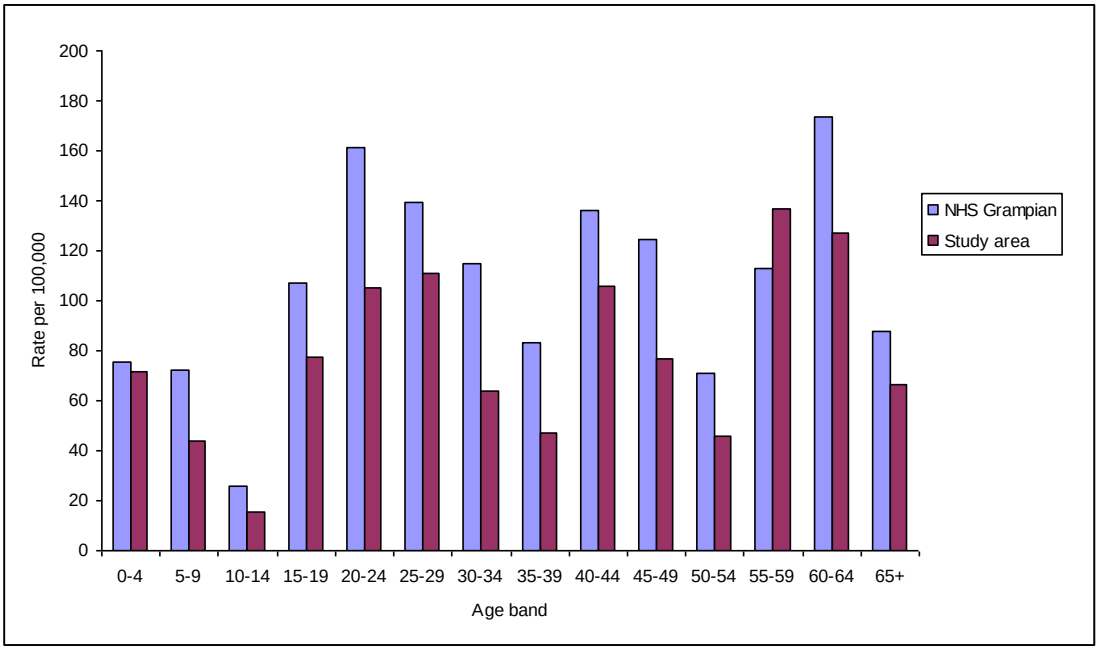


Figure 6: Rates per 100,000 for female *Campylobacter* cases in Grampian NHS Board area and cases participating in the study



4.2.3 Residence of cases and controls and population of the study area

The population of Aberdeen City and Aberdeenshire is 211,250 and 227,200 respectively therefore population of the study area is 439,450. 48.2% of the population is resident in Aberdeen City and 51.8% is resident in Aberdeenshire.

Controls were selected at random from the CHI for the population of Aberdeen City and Aberdeenshire. The selection did not use any matching based on local authority or postcode and therefore should be representative of the study population. 50.4% of controls were resident in Aberdeen City and 49.6% in Aberdeenshire, compared to 48.2% and 51.8% respectively for the study population. There was no significant difference between the proportion of controls resident in Aberdeen City and Aberdeenshire and the total study population (Table 4).

Overall 57.9% cases were resident in Aberdeenshire compared to 49.6% of controls. Cases were significantly more likely to be resident in Aberdeenshire than controls (Chi-square 15.54, $p < 0.001$) (Table 4)

Cases were also significantly more likely to be resident in Aberdeenshire than the total study population (Chi-square 11.75, $p < 0.001$).

Table 4: Cases and controls resident in Aberdeen City and Aberdeenshire

Local Authority area	Case (%)	Control (%)
Aberdeen City	332 (42.1)	957 (50.4)
Aberdeenshire	457 (57.9)	941 (49.6)
Total	789	1898

4.2.4 Deprivation category of cases and controls

For three cases and ten controls it was not possible to assign a deprivation category to their post code.

Deprivation category one represents the greatest affluence and deprivation category seven - the areas of greatest deprivation.

There were no cases or controls resident in a deprivation category seven postcode, this was due to no such postcodes in the study area, rather than a lack of participation among cases or controls in such areas.

The overall distribution of cases and controls by deprivation category was similar (Figure 7). Although a greater proportion of controls were resident in deprivation category one areas compared to cases, 25.5% and 21.2% respectively. This was reversed in deprivation category two areas, with 35.7% cases and 28.8% controls (Appendix Table 6).

4.2.5 Comparison to the population of the study area and Scotland

In Aberdeen City and Aberdeenshire there are no postcode sectors which are deprivation category 7, while overall within Scotland 7% of the population are resident in a category 7 area. Within Aberdeenshire there were no postcode sectors of deprivation category 5 or 6 (Appendix Table 7).

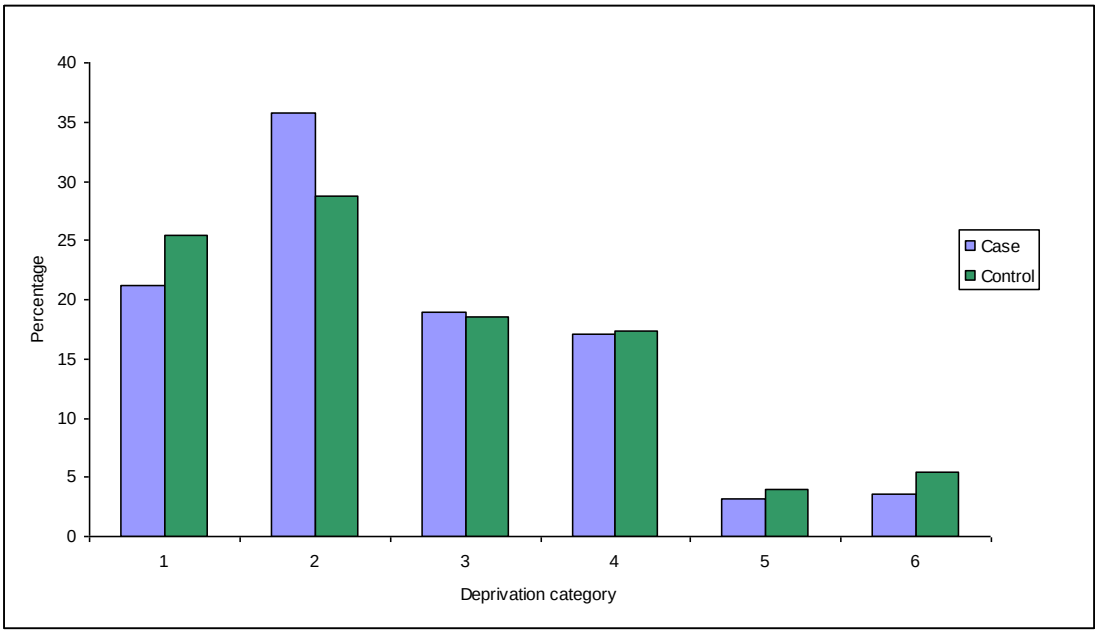
In both Aberdeen City and Aberdeenshire a greater proportion of the population is resident in the two most affluent areas, compared to the population of Scotland. 24% of the population of Aberdeenshire and 16% of the population of Aberdeen City is resident in a deprivation category one postcode compared to only 6% of the population of Scotland (Appendix Table 7).

When considering cases and controls resident in Aberdeen City, 22% of cases and 23% of controls were resident in deprivation category one areas, while overall only 16% of Aberdeen City are resident in such postcodes. For category 2, 3 and 4 areas the proportion of cases, controls and city population in these areas was broadly similar. Only 8% of cases and 11% of controls were resident in category six areas compared to 14% overall for the city.

When considering cases and controls resident in Aberdeenshire, 21% cases and 28% controls were resident in category one areas and for category two areas it was 43% and 33% respectively. For the population of Aberdeenshire 24% and 34% were resident in category one and two areas, respectively (Appendix Table 7)

Cases and controls were also considered by the Scottish Index of Multiple Deprivation (SIMD) score (Appendix Table 8). SIMD was used when considering deprivation in the adjusted odds ratios, by considering SIMD divided into quartiles. This analysis showed the risk of infection increased in areas of least deprivation compared to the most deprived quartile (Table 44).

Figure 7: Deprivation Category for cases and controls



4.2.6 Occupation group of cases and controls

The 'occupation field' on the questionnaire was a free text field which was used to code the occupations to the groups of the Standard Occupational Classification 2000 Vol 1. For 20 cases and 34 controls it was not possible to code the occupation, as information was either missing or was insufficient for coding. Coding was done as accurately as possible with the information on the questionnaires, and any errors in coding should be consistent between cases and controls. (Appendix Table 9)

The retired accounted for the largest single group representing 16.6% of cases and 15.7% of controls. The proportion of cases and controls in each of the occupation groups was similar. The largest difference was among those coded as 'Professional Occupations' who accounted for 7.2% of cases and 10.2% of controls.

Occupational contact with animals was derived from the free text field on occupation. As there was no specific question on occupational contact with animals, and jobs were coded for those that were likely to involved animal contact, for example vets, veterinary nurses and farmers.

Among adult cases and controls 1.5% and 1.7% respectively had a job likely to involve contact with animals, this was not significantly different (odds ratio 0.85 (0.39 to 1.85), p = 0.67) (Appendix Table 10). There was no significant

difference between adult cases and controls in having job that was likely to involve contact with raw meat, 1.9% and 1.5% respectively (Appendix Table 11).

4.2.7 Year and Month of participating in the study

In two months during the study period: October and November 2005, the number of questionnaires received from cases exceeded the number received from controls. October 2005 was the period between the pilot study and the main study and no control packs were sent out. The returned questionnaires represent those sent out during the pilot study and returned in October. November 2005 was the first month of the main study and control packs were not sent out until towards the end of the month (Figure 8).

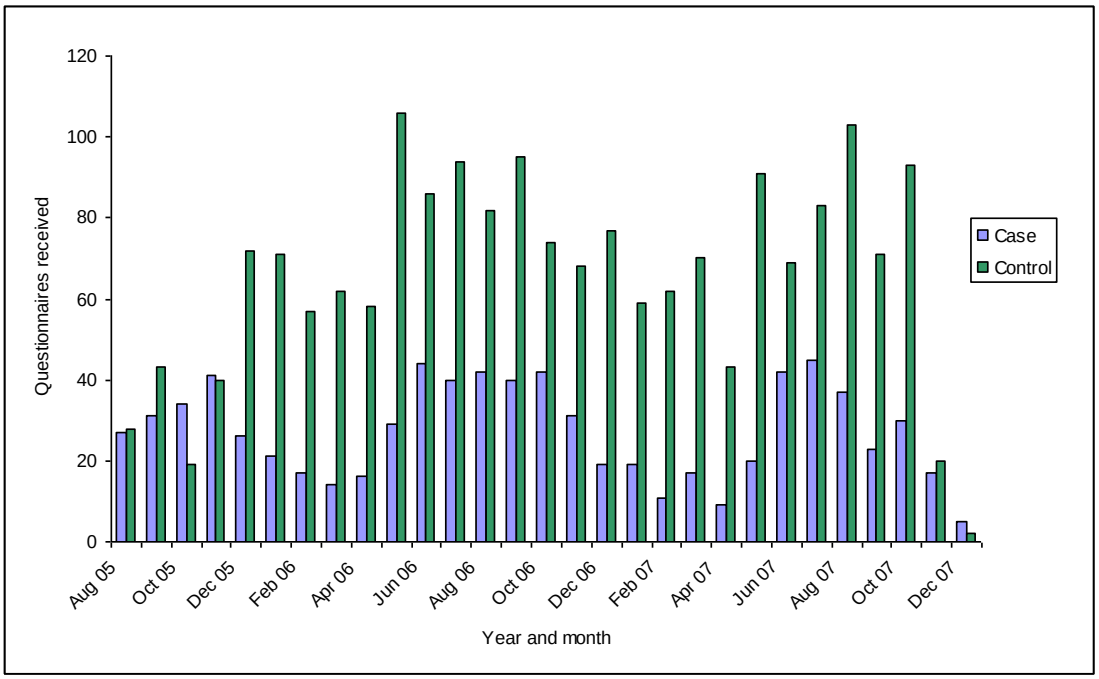
The low number of questionnaires received from both cases and controls in November and December 2007 represents the end of the study. No new questionnaires were sent out and only those already posted to participants in previous months were being returned.

During the pilot study, the matching frequency was 1.03:1 and 1.38:1 for August and September 2005 respectively. The number of controls invited to participant was increased during the main study. In the 23 months between December 2005 and October 2007, a matching frequency of 2:1 or greater was achieved in 18/23 (78%) of the months. The five months in which it was below 2:1 were June and August 2006 in both of which the frequency was 1.95:1, October 2006 with frequency 1.76:1, June and July 2007, 1.64:1 and 1.84:1 respectively (Figure 8).

April 2007, was the month with the fewest cases participating with only nine compared to 43 controls. There were seven months in which more than 40 cases participated June to October 2006 and June and July 2007.

The seasonal increase in cases appeared greater in 2006 than 2007. Between May and October 2006, 237 cases participated compared to 197 during the same period in 2007, a decline of 40 (16.8%). For the same months the number of controls remained similar, with 537 and 510 in 2006 and 2007 respectively, a decline of 27 (5.0%) (Figure 8).

Figure 8: Year and month questionnaire was received at HPS



4.3 Length of time between onset, completing the questionnaire and completeness of the questionnaire

4.3.1 Length of time between onset and completing questionnaire for cases

The length of time was calculated for cases between the onset date on the questionnaire and the date the questionnaire was completed by the case. This information was available for 97% (766) of cases. The length of time ranged from 3 to 123 days (Figure 9). Nine days was the most commonly identified duration between onset and completing the questionnaire reported by 11.7% (90/766) cases. The mean length of time between onset and completing the questionnaire was 16.0 days.

68% (538) questionnaires were completed 14 days or less after the date of onset, 16.6% (131) were completed between 15 and 28 days after onset and 12.3% (97) were completed 29 or more days after onset.

There was no significant difference between children and adults in the average time between onset and completing the questionnaire, 16.4 and 15.9 days respectively (t-test, df = 762, p = 0.692).

There was no significant difference between males and females in the average time between onset and completing the questionnaire, 15.9 and 16.2 days respectively (t-test, df = 764, p = 0.778).

4.3.2 Association between completeness of questionnaires, time between onset and completing questionnaire for cases

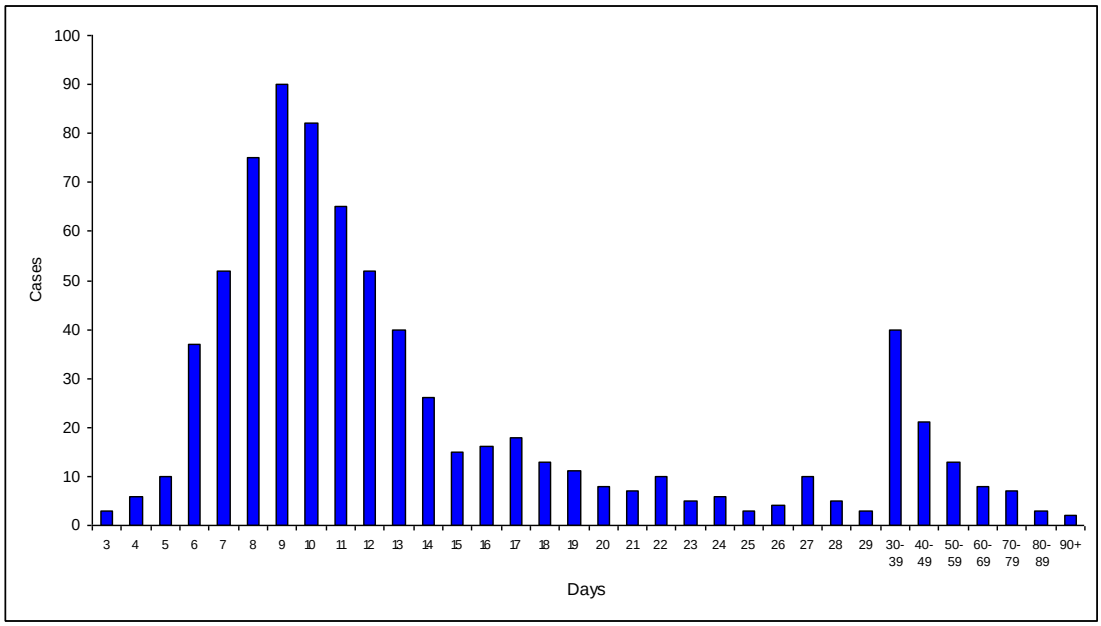
Responses were grouped for those that completed the questionnaire in 14 days or less from the date of onset and those who completed it more than 14 days from the date of onset.

69% (538) of cases completed the questionnaire within 14 days or less from their date of onset, 29% (228) completed the questionnaire more than 14 days after their date of onset (Figure 9). As mentioned above for 23 cases (3%) it was not possible to determine the length of time between onset and completing the questionnaire due to the lack of one or other date.

For most questions, the rate of missing answers was similar between those participants completing the questionnaire within 14 days of their onset and those completing it more than 14 days after their onset. However, for eating outside the home answers were missing for 2.0% of cases completing the questionnaire within 14 days compared to 6.6% for those completing it after more than 14 days. Similarly, for eating chicken prepared at home these values were 5.4% and 9.2% respectively (Appendix Table 12).

For some questions participants had the option of answering questions as ‘not known’ or ‘not sure’. The percentage selecting this option was generally similar for those completing the questionnaire within 14 days and those completing it more than 14 days after onset. However, there were some questions where this option was higher among those completing it after more than 14 days, in particular for drinking bottled water, eating raw vegetables washed in tap water, eating salads washed in tap water, eating fruit washed in tap water and eating salads/raw vegetables at home (Appendix Table 12).

Figure 9: Days between onset and completing the questionnaire for cases (n=766)



(Note: Change in scale of graph, after 29 days, to allow the data to be presented on one graph)

4.4 Clinical presentation of infection

4.4.1 Symptoms reported by cases and controls

One case did not record which symptoms they suffered from. This case was still included in the study as they were microbiologically positive for *Campylobacter,* which was the criterion for inviting cases to participate.

The most frequently reported symptom among cases was diarrhoea reported by 99.2% (783) cases. Five cases responded that they did not suffer from diarrhoea, all five of whom reported abdominal pain and two of the five reported bloody stools (Table 5)

The proportion of cases reporting abdominal pain was also high, reported by 91.8% (724). 18 cases responded as 'not sure' to question regarding abdominal pain, 66.7% (12) were children aged 4 years or under, where a parent was completing the questionnaire on the child's behalf.

Vomiting was reported by 36.9% (291) of cases. Bloody stools were the least common of the four symptoms covered by the questionnaire reported by 31.2% (246).

Only 5.3% (42) of cases reported suffering from only one of the four symptoms on the questionnaire. 11.9% (94) reported suffering from all four symptoms (Table 6).

Three controls did not complete the section on suffering from any symptoms.

Among controls abdominal pain was the most common symptom reported by 10.9% (208). Bloody stools were the least common symptom among controls reported by 1.2% (23) (Table 5).

Overall 16.1% (306) of controls reported suffering from at least one symptom in the five days prior to completing the questionnaire (Table 6).

4.4.2 Admission to hospital

Admission to hospital was defined as 'any nights spent in hospital as a result of the illness', 10.7% (84) of cases were admitted to hospital. Admission to hospital varied between the age bands from 5.4% for those aged 30-39 years to 33.3% for these aged 80 years and above, however the total number in this group was relatively small with only 18 cases, 6 (33.3%) of whom were admitted to hospital. The general trend was for the higher rates of hospitalisation to be observed at the two ends of the age spectrum (Figure 10).

Cases who were admitted to hospital were significantly more likely to report bloody stools ($p = 0.004$) and vomiting ($p=0.007$) than those not admitted. There was no significant difference in the reporting of diarrhoea or abdominal pain between cases admitted and not admitted to hospital (Tables 7 & 45). Overall cases who were admitted to hospital reported more symptoms than those not admitted ($p = 0.017$) (Table 8).

4.4.3 Duration of illness

At the time of completing the questionnaire 35.0% (276) of cases were still ill, 63.0% (504) reported they had recovered. For 479 cases who had recovered, information was available for the duration of illness, with both onset date and date symptoms had stopped.

Duration of illness ranged from 0 to 70 days. Seven days was the most frequently reported duration 11.2% (88/479) cases. For 53.2% (255/479) of cases the duration of their illness was 1 to 7 days, 36.1% (173/479) were ill for 8 to 14 days, for 10.6% (51/479) their illness lasted for 15 or more days (Figure 11).

Overall the mean duration of illness was 9.00 days (std deviation 6.73). The total number of days of illness reported by the 479 cases was 4309 days, the equivalent to 11.8 years.

There was no significant difference in the duration of illness between adults and children, 9.0 and 9.1 days respectively (t-test $t = 0.175$, $df = 477$, $p = 0.861$).

When considering the adult cases only, there was a significant difference in the duration of illness between males and females. The duration of illness was significantly longer in females than males, 9.7 days and 8.3 days respectively (t-test $t=2.156$, $df = 403$, $p = 0.032$).

There was no significant difference between the genders in the duration of illness among the child cases, with 9.6 days for females and 8.8 days for males (t-test t = 0.354, df = 72, p = 0.725).

The mean duration of illness was significantly longer for those admitted to hospital than those not admitted 12.4 days compared to 8.7 days (t-test, t = 3.206, df = 476, p = 0.001). Among cases admitted to hospital duration of illness ranged from 3 to 70 days.

Table 5: Symptoms reported by cases and controls

Symptom	Case	Control
	Number (%)	Number (%)
Diarrhoea	783 (99.2)	177 (9.3)
Bloody stools	246 (31.2)	23 (1.2)
Abdominal pain	724 (91.8)	208 (11.0)
Vomiting	291 (36.9)	53 (2.8)

Table 6: Number of symptoms reported by cases and controls

Number of symptoms	Case Number (%)	Control Number (%)
0	0	1589 (83.7)
1	42 (5.3)	191 (10.1)
2	329 (41.7)	79 (4.2)
3	323 (40.9)	31 (1.6)
4	94 (11.9)	5 (0.3)
Not recorded	1 (0.1)	3 (0.2)
Total	**789**	**1898**

Figure 10: Percentage of cases admitted to hospital by age band

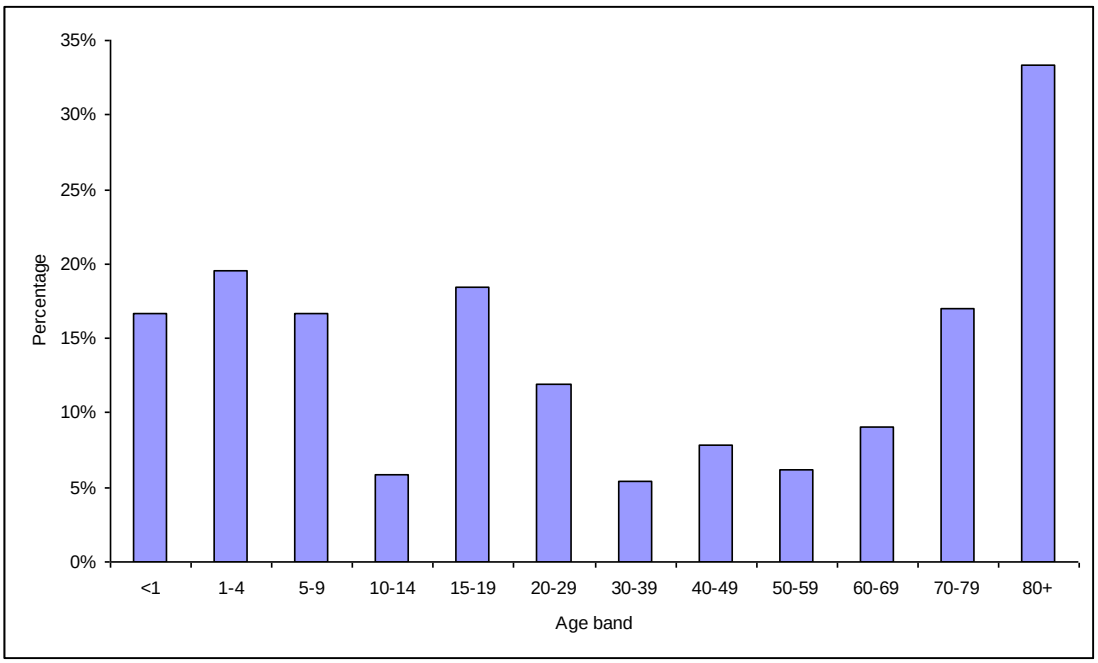


Table 7: Symptoms reported by cases admitted to hospital and cases not admitted

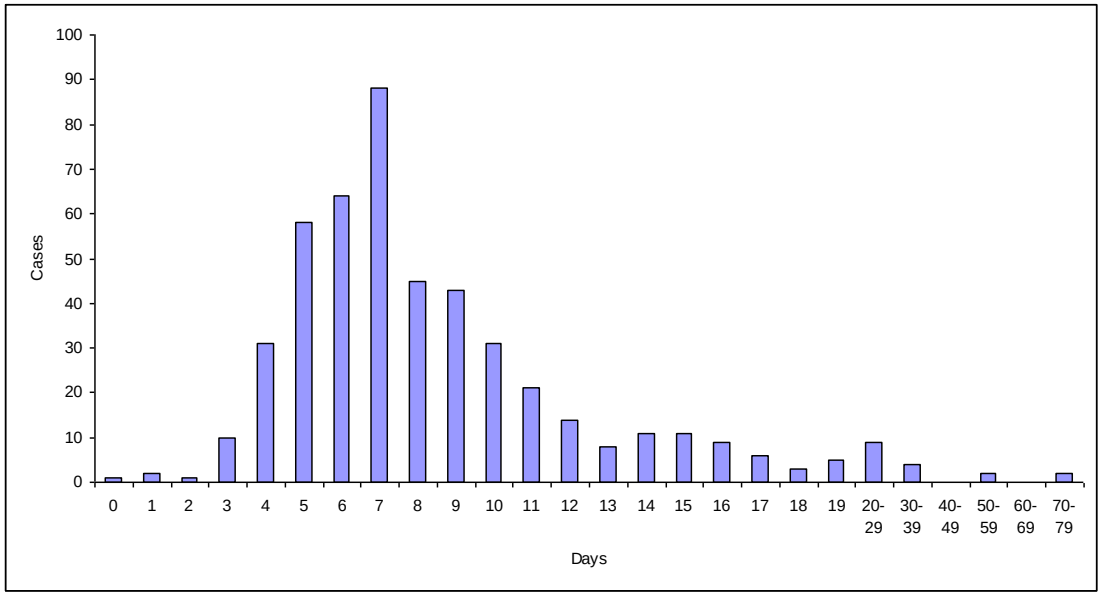
Symptom	Not admitted Number (%)	Admitted Number (%)	Chi Square	P
Diarrhoea	697 (99.4)	83 (98.8)	0.455	0.500
Bloody stools	205 (29.2)	39 (46.4)	8.463	0.004
Abdominal pain	646 (92.1)	75 (89.3)	0.319	0.572
Vomiting	248 (35.4)	42 (50.0)	7.403	0.007

Table 8: Total number of symptoms reported, for those admitted and not admitted to hospital

Number of symptoms	Not admitted Number (%)	Admitted to hospital Number (%)
1	41 (5.8)	1 (1.2)
2	302 (43.1)	27 (32.1)
3	280 (39.9)	40 (47.7)
4	78 (11.1)	16 (19.1)
Total	**701**	**84**

Overall cases who were admitted to hospital reported more symptoms than cases not admitted, $X^2 = 10.198$, df = 3, p = 0.017

Figure 11: Duration of illness for cases that provided information on onset date and date when symptoms stopped



(Note: Change in scale of graph, after 19 days, to allow the data to be presented on one graph)

4.5 Other members of the same household reported with similar symptoms

4.5.1 At least one other member of the same household reported with similar symptoms

81.7% (645) cases lived in a household with at least one other person, 5.8% (46) cases reported being the only person in the household and this information was missing for 12.4% (98) cases.

13.6% (88) of cases who lived in the same household as others, reported that at least one other person in their household had similar symptoms. These 88 cases, reported a total of 114 other members of the same household were ill with similar symptoms (Appendix Table 14).

Child cases were significantly more likely to report that at least one other person in the household was ill with similar symptoms than adult cases, 28.3% compared to 11.1% (Chi square 20.94, $p < 0.001$) (Appendix Table 15).

4.5.2 Two or more laboratory confirmed cases in the same household

There were nine households in which more than one laboratory confirmed case of *Campylobacter* infection participated in the study. In these nine households, eight contained two confirmed cases and one three cases. 66.7% (6) of the households with more than one confirmed case participating in the study included one or more children who were positive for *Campylobacter* (Appendix Table 16).

For five of these households the dates of onset were within seven days of each other. For the other four households the difference between the dates of onsets in the same household ranged from 16 to 64 days.

The 19 cases reported from households with more than one confirmed case participating in the study, represent 2.4% of cases in the study.

4.6 Exposure differences between cases and controls

For the analysis of exposure differences between cases and controls, controls were selected to exclude all those who reported any of the four symptoms typical of *Campylobacter* infection (diarrhoea, bloody stools, abdominal pain or vomiting). 306 controls reported suffering from at least one of the symptoms which would be compatible with *Campylobacter* infection, additionally there were three controls who did not answer these questions and were therefore excluded from this section of the analysis. As a result of the exclusion of these controls, 1589 ‘well’ controls were included in the analysis presented below on the exposure characteristics between cases and controls (Table 9). This analysis was also repeated using all controls (Table 41). The analysis was also conducted using only Aberdeenshire data, and conducted in this area using both all controls and ‘well’ controls only (Tables 42 & 43).

After excluding controls which had symptoms compatible with *Campylobacter* infection the overall matching frequency for cases and 'well' controls was 1:2.

Table 9: Cases and controls included in analysis of exposure differences

	Case	Controls
Child	105	245
Adult	684	1344
Total	**789**	**1589**

4.7.1 Medicines taken regularly by cases and controls

The questionnaire contained questions on particular groups of medicines taken regularly in the 28 days before onset for cases, or 28 days before completing the questionnaire for controls.

Due to the very small number of children taking any of the medicines, the analysis of medicines taken regularly was restricted to adults.

There was no significant difference between adult cases and controls in the use of either antacids or antibiotics (Appendix Tables 17 &18).

The use protein pump inhibitors/H_2 receptor antagonists e.g. omeprazole, cimetidine or ranitidine was reported by significantly more adult cases than controls, 18.4% and 8.7% respectively (Table 10) (odds ratio 2.40 (1.81 to 3.17), $p < 0.001$), (adjusted odds ratio 2.732 (2.052-3.639) (Table 44).

As the questionnaire grouped omeprazole, cimetidine and ranitidine together in the same question, it was not possible to investigate any specific association with particular drugs within this group.

The use of omeprazole, cimetidine or ranitidine was the only one of the three medicine groups on the questionnaire to show a significant association with being a case of *Campylobacter* infection.

4.7.2 Association between medicines taken regularly by cases and admission to hospital

For the 684 adult cases in the study, analysis was undertaken to investigate the use of medicines in the 28 days prior to onset and their subsequent admission to hospital.

There was no significant difference in the use of antacids by adult cases and admission to hospital (Appendix Table 19).

The use of antibiotics in the 28 days prior to onset was reported by 14.7% (10) of adult cases who were admitted to hospital, but only 5.7% (35) of adult cases not admitted to hospital (Table 11). Adult cases admitted to hospital were significantly more likely to report the prior use of antibiotics (Chi square 8.45, $p = 0.0036$). However there is no means of verifying that the antibiotics had actually been taken prior to onset, rather than cases mistakenly referring

to antibiotics that they have been prescribed in response to the *Campylobacter* infection.

The use of omeprazole, cimetidine or ranitidine was reported by 27.9% (19) of adult cases who were admitted to hospital and 17.4% 107) of cases who were not admitted to hospital (Table 12). Adult cases admitted to hospital were significantly more likely to report the prior use of omeprazole, cimetidine or ranitidine (Chi square 4.68, p = 0.0305).

The prior use of antibiotics and omeprazole, cimetidine or ranitidine by cases were both significantly association with subsequent admission to hospital with *Campylobacter* (Tables 11 & 12), but only the taking of omeprazole, cimetidine or ranitidine was significantly associated with acquiring *Campylobacter* infection (Tables 10 & 44). However, it should also be taken into consideration that the number of cases in some of these groups was relatively small, and there may have been misinterpretation of the antibiotics question.

Table 10: Adult, cases and controls taking Omeprazole (Losec), Cimetidine (Tagamet) or Ranitidine (Zantac)

Omeprazole, Cimetidine, Ranitidine	**Case (%)**	**Control (%)**
No	543 (79.4)	1198 (89.1)
Yes	126 (18.4)	116 (8.6)
Missing	15 (2.2)	30 (2.2)
Total	**684**	**1344**

Odds ratio = 2.40 (1.81 to 3.17), p < 0.001

Table 11: Use of antibiotics and admission to hospital

Use of antibiotics	**Admission to hospital**	
	No (%)	**Yes (%)**
No	545 (89.1)	53 (77.9)
Yes	35 (5.7)	10 (14.7)
Missing	32 (5.2)	5 (7.4)
Total	**612**	**68**

Chi square = 8.45, p = 0.0036

Table 12: Use of Omeprazole (Losec), Cimetidine (Tagamet) or Ranitidine (Zantac) and admission to hospital

Use of Omeprazole etc	**Admission to hospital**	
	No (%)	**Yes (%)**
No	494 (80.7)	47 (69.1)
Yes	107 (17.4)	19 (27.9)
Missing	11 (1.8)	2 (2.9)
Total	**612**	**68**

Chi square = 4.68, p = 0.0305

4.8.1 Travel outside the study area

Travel outside the study area was defined as an overnight stay outside the study area (Aberdeenshire or Aberdeen City) in the 14 days before onset, or for controls the 14 days prior to completing the questionnaire.

Travel outside the study area was reported by 34.6% (273) of cases, compared to 20.8% (330) of controls (Table 13). Cases were significantly more likely to have had an overnight stay outside the study area (odds ratio 2.03 (1.67 to 2.47), $p < 0.001$) (adjusted odds ratio 2.133 (1.750-2.599)) (Table 44).

When analysed separately for children and adults, cases in both groups were significantly more likely to have had an overnight stay outside the study area than the corresponding controls. There was no significant difference between children and adults in the value of the odds ratio related to an overnight stay (Appendix Table 20).

4.8.2 Locations associated with travel outside the study area

Some participants did not provide details of the country visited, for example wrote 'on holiday' in the free text field for details of place visited.

2.7% (21) of cases and 0.9% (15) of controls are reported as having travelled to more than one country, this group could have included participants with travel within Scotland and England & Wales, as these were coded separately in the study, as well as those who travelled to more than one country overseas.

For 46.5% (127) cases with a history of travel, their travel was within Scotland (95) or England & Wales (32), therefore 146 cases had a history of travel overseas, accounting for 53.4% of cases with any travel history and 18.5% of all cases (Table 14)

For 63.0% (208) of controls with a history of travel, their travel was within Scotland (160) or England & Wales (48), therefore 122 controls had a history of travel overseas, accounting for 36.9% of controls with any travel history and 7.7% of all controls (Table 14).

Overall 53.4% cases with an overnight stay outside the study area and accounting for 18.5% of all cases, had an overnight stay overseas, compared to 36.9% of controls with an overnight stay outside the study area and accounting 7.7% of all controls. Therefore overall 18.5% of all cases had been overseas compared to just 7.7% of all controls (adjusted odds ratio 3.378 (2.528-4.514)) (Table 44).

Interestingly, 34.8% of cases with on overnight stay outside the study area reported this was within Scotland, compared to 48.5% of controls with an overnight stay outside the study area (Table 14)

1.1% (3) of cases with an overnight stay outside the study area reported working offshore. Offshore working was reported by 3.9% (13) of controls with an overnight stay outside the study area (Table 14).

For 5 cases and 3 controls it was not possible to assign a continent visited, as these participants either provided no details of the location, e.g. ‘on holiday’ or were on a cruise and did not specify the countries visited.

The largest difference in the continents visited was observed with travel to Asia reported by 11.4% of cases with an overnight stay outside the study area, compared to 3% of such controls.

Spain was the overseas country the most frequently visited by cases, controls and the population of Scotland (Table 15). Turkey was the second most frequently visited country for cases, accounting for 5.5% of cases with any overnight stay outside the study area, but was only visited by 0.9% of controls with any history of travel and did not appear in the top ten countries reported for visits by the Scottish population (data for Scotland from the International passenger survey and travel trends, Travel trends 2006). USA and Republic of Ireland were both in the top five countries visited by the Scottish population, but neither were in the top five countries reported by cases, while the Republic of Ireland was in the top five countries visited by controls.

4.8.3 Association between travel outside the study area and month of participating in the study

The proportion of cases reporting an overnight stay outside the study area each month ranged from 14.2% to 70.3%, for controls it ranged from 6.1% to 36.5% (Figure 12).

The first month of the pilot study (August 2005) was the month with the highest percentage of cases reporting an overnight stay outside the study area (70.3%). This may in part be a data artefact as no cases would have returned questionnaires at the start of the month, for questionnaires sent out the previous month, as would have happened in August 2006 and August 2007. After August 2005, October 2006 had the greatest proportion of cases with an overnight stay accounting for 54.7% of case questionnaires received that month, in comparison an overnight stay was reported by 15.1% of controls received that month.

In March 2006, 14.2% of cases reported an overnight stay outside the study area. This was the lowest proportion for any month, during the same month 17.6% of controls reported an overnight stay outside the study area.

In only three months of the study: March 2006, November 2006 and June 2007, did a greater proportion of controls report an overnight stay outside the study area than cases.

Among cases who reported on overnight stay outside the study area 11.7% (32) reported they were aware of someone else with a similar illness where they were staying, e.g. same hotel. From the information collected, it was not

possible to determine if any of these persons with similar symptoms, were also positive for *Campylobacter* or were suffering from another gastro-intestinal illness.

4.8.4 Association between travel outside the study area and reporting other members of the same household to be ill with similar symptoms

Of the 641 cases that reported others living in the same household, information was available for 640 cases on whether they had had an overnight stay outside the study area.

Among the 405 cases which do not live alone and had no overnight stay outside the study area, 11.6% (47) reported that other members of their household had similar symptoms. Among the 235 cases who do not live alone and who had an overnight stay outside the study area, 17.4% (41) reported that other members of their household had similar symptoms. This represented a significant difference (Chi square = 4.28, p = 0.038) (Table 16).

From the information collected in the study, it is unknown whether the others reported with similar symptoms were ill with *Campylobacter*, or if they had another gastrointestinal illness. Similarly it is unknown if those reported to be ill had the same overnight stay outside the study area as the case.

Table 13: Cases and controls with an overnight stay outside the study area

Overnight stay outside study area	**Case (%)**	**Control (%)**
No	512 (65.9)	1258 (79.2)
Yes	273 (34.6)	330 (20.8)
Missing	4 (0.5)	1 (0.1)
Total	**789**	**1589**

Odds ratio = 2.03 (1.67 to 2.47), p < 0.001

Table 14: Cases and controls by travel country

(For those who reported an overnight stay outside the study area)

Travel country	Cases	Controls
	Number (%)	Number (%)
Australia	0	1 (0.3)
Canada	0	2 (0.6)
Cruise	2 (0.7)	2 (0.6)
Cyprus	2 (0.7)	0
Eastern Europe	7 (2.6)	2 (0.6)
England and Wales	32 (11.7)	48 (14.5)
France	7 (2.6)	5 (1.5)
Germany	0	2 (0.6)
Greece	0	2 (0.6)
India	4 (1.5)	0
Ireland	1 (0.4)	5 (1.5)
Italy	1 (0.4)	3 (0.9)
Middle East	3 (1.1)	0
More than 1 country	21 (7.7)	15 (4.5)
N/K	3 (1.1)	3 (0.9)
North Africa	11 (4.0)	5 (1.5)
Offshore	3 (1.1)	13 (3.9)
Other Europe	8 (2.9)	9 (2.7)
Portugal	5 (1.8)	5 (1.5)
Rest of Asia	5 (1.8)	6 (1.8)
Scotland	95 (34.8)	160 (48.5)
South Africa	0	1 (0.3)
South America	2 (0.7)	0
Spain	43 (15.8)	28 (8.5)
Turkey	15 (5.5)	3 (0.9)
USA	3 (1.1)	10 (3.0)
Total	**273**	**330**

The above table includes only locations that were coded to an individual country, some of the less frequently visited countries were coded to groups of countries e.g. Eastern Europe or North Africa

Table 15: Comparison of top five overseas countries visited by cases, controls and the top ten countries visited by the Scottish population, using data from Travel Trends 2006.

Cases	Controls	Travel trends data (Visits thousands)
Spain (43)	Spain (28)	Spain (1,470)
Turkey (15)	France (5)	France (417)
France (7)	Ireland (5)	USA (378)
Portugal (5)	Portugal (5)	Irish Republic (236)
India (4)	Italy (3)	Italy (193)
	Turkey (3)	Greece (180)
		Portugal (161)
		Germany (151)
		Netherlands (132)
		Poland (128)

.

Figure 12: Percentage of cases and controls who reported an overnight stay outside the study area by study year and month

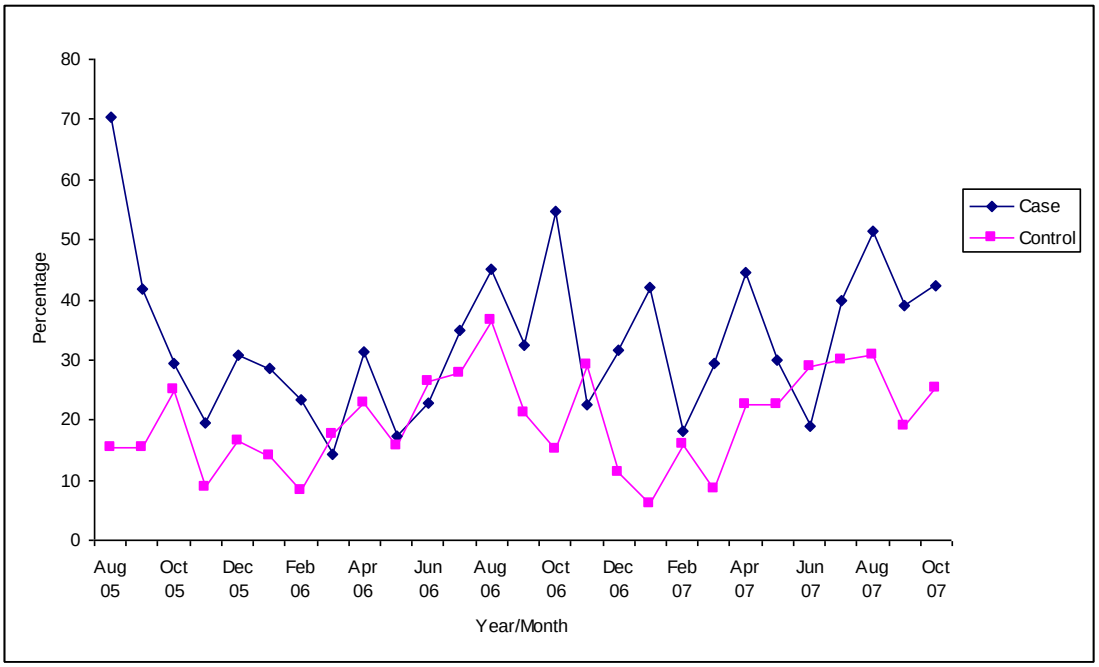


Table 16: Overnight stay outside the study area and reporting other members of the household to be ill with similar symptoms

Selected for the 641 cases that reported that others lived in the same household as the case, for 640 of these cases information was available on whether they had had an overnight stay outside the study area.

Number of others in household also ill	Overnight stay outside study area	
	No (%)	Yes (%)
0	358 (88.4)	194 (82.6)
1	40 (9.8)	29 (12.3)
2	5 (1.2)	7 (3.0)
3	2 (0.5)	5 (2.1)
Total	**405**	**235**

4.9 Animal contact

4.9.1 Pet animals

Having a pet at home was reported by 50.6% (399) of cases, compared to 45.6% (724) of controls (Table 17). Cases were significantly more likely than controls to report having a pet at home (odds ratio 1.23 (1.03 to 1.46), p = 0.0195). As the odds ratio was only 1.23, this represents only a small risk.

When the responses for having a pet at home are considered separately for children and adults (Appendix Table 21), there remains a statistical significant difference for having a pet a home for adults, reported by 50% (342) of adult cases, compared to 43.6% (586) of adult controls (odds ratio = 1.30 (1.08 to 1.57) p = 0.0054), again this represents only a small risk. However, there was no statistical significant difference for children between cases and controls in having a pet at home, reported by 54.3% (57) of child cases and 56.3% (138) of child controls, (odds ratio = 0.91 (0.56 to 1.48) p = 0.695). Overall having a pet only just represented a significant risk in the adjusted odds ratio 1.238 (1.033 to 1.483) (Table 44).

4.9.2 Types of pet

Within the data for type of pet reported, many participants reported having more than one type of pet, or having more than one dog or cat, etc. (Appendix Table 22). The study did not seek to collect information on the actual number of pets within the household only the types of pet.

Among both cases and controls with a pet a home, a dog was the most common, reported by 59.1% (236) of cases and 49.6% (359) of controls. A cat was the second most common reported by 44.3% (177) of cases and 44.1% (320) controls with a pet.

4.9.3 Pets reported to be ill with diarrhoea or vomiting

For those participants who reported having a pet in the household, 5.5% (22) of cases reported a pet that had been ill with diarrhoea or vomiting, compared to 3.0% (22) of controls. This difference was statistically significant (p = 0.0355), however the total numbers involved were relatively small.

4.9.4 Contact with farm animals

Participants were asked if they had had any contact with farm animals in the five days before onset or completing the questionnaire. The questionnaire asked the participant to provide details of the type of farm animal(s) in a free text field. During the preparation of the database, information given in the 'any other pet field' and 'type of farm animal' was checked and where necessary the data/responses amended accordingly. For example, where a case reports sheep under category 'any other pet', this was coded as being a farm animal, and response 'yes' to question regarding farm animals was applied rather than the animal being recorded as a pet.

Contact with farm animals was reported by 9.5% (75) of cases, compared to 6.2% (99) of controls (Table 18). Cases were significantly more likely to report contact with farm animals than controls (odds ratio 1.50 (1.08 to 2.09), p = 0.00115), however when the adjusted odds ratios were determined this was no longer a risk factor (1.320 (0.939-1.855)) (Table 44).

Contact with farm animals was considered separately for adults and children (Appendix Table 23). 7.3% (50) of adult cases and 5.7% (77) of adult controls reported contact with farm animals this was not significantly different (odds ratio 1.23 (0.83 to 1.81), p = 0.282).

Among child cases 23% (25) reported contact with farm animals, compared to 9.0% (22) of child controls, this was significantly different (odds ratio 3.05 (1.54 to 6.04), p = 0.000405). However, the relatively small number of child cases and controls means the confidence interval for the odds ratio of 3.05 for children ranged from 1.54 to 6.04.

Having contact with farm animals did not make a significant difference as to whether other members of the same family were ill with similar symptoms (Appendix Table 24).

Table 17: Cases and controls who report having a pet at home

Pets at home	**Case (%)**	**Control (%)**
No	386 (48.9)	859 (54.1)
Yes	399 (50.5)	724 (45.6)
Missing	4 (0.5)	6 (0.4)
Total	**789**	**1589**

Odds ratio = 1.23 (1.03 to 1.46) p = 0.0195

Table 18: Cases and controls who reported any contact with farm animals in the 5 days before onset or completing the questionnaire

Contact with farm animals	**Case (%)**	**Control (%)**
No	571 (72.40	1133 (71.3)
Yes	75 (9.5)	99 (6.2)
Missing	143 (18.1)	357 (22.4)
Total	**789**	**1589**

Odds ratio = 1.50 (1.08 to 2.09) p = 0.0115.

4.10. Eating food outside the home

Information was collected on eating out or food obtained from 'carry out' facilities in the five days before onset or completing the questionnaires.

Eating outside the home was reported by 59.3% (468) of cases, compared to 46.6% (741) of controls (Table 19). Cases were significantly more likely to report eating outside the home than controls (odds ratio 1.77 (1.48 to 2.12), $p < 0.001$).

4.10.1 Eating chicken outside the home

From the details provided on types of foods eaten outside the home a derived field was created in the database, for those that had eaten outside the home to record whether chicken was consumed. Some participants answered 'yes' to 'eating outside the home', but then did not provide details of the types of foods consumed. For others, from the details provided it was not possible to determine if the meal contained chicken, for example, the answer was 'food from buffet', 'curry' or 'sandwiches'.

For cases and controls that reported eating outside the home, and for which information on chicken consumption outside the home was available, eating chicken was reported by 37.8% (177) of cases and 28.3% (210) of controls (Table 20). Cases from this group were significantly more likely to have eaten chicken out than controls (odds ratio = 1.52 (1.14 to 2.01), $p = 0.0026$).

22.4% (177) of all cases and 13.2% (210) of all controls ate chicken outside the home. 60.2% (472) of all cases and 72.5% (1152) of all controls either did not eat outside the home, or when eating outside the home did not report eating chicken (Table 21). This was a significant difference (odds ratio = 2.04 (1.62 to 2.58), $p < 0.001$). Therefore, eating chicken prepared outside the home appeared to be a risk factor for *Campylobacter* infection and was also a significant risk factor in the adjusted odds ratio 2.118 (1.677 to 2.676) (Table 44).

Table 19: Participants who reported eating food outside the home or from 'carry out' facilities

Ate food outside the home or from "carry outt"	**Case (%)**	**Control (%)**
No	297 (37.6)	832 (52.4)
Yes	468 (59.3)	741 (46.6)
Missing	24 (3.0)	16 (1.0)
Total	**789**	**1589**

Odds ratio = 1.77 (1.48 to 2.12), $p < 0.001$

Table 20: Participants who reported consuming chicken while eating outside the home.

Ate chicken outside the home	Case (%)	Control (%)
No	178 (38.0)	320 (43.2)
Yes	177 (37.8)	210 (28.3)
Not specified	91 (19.4)	159 (21.5)
Missing	22 (4.7)	52 (7.0)
Total	**468**	**741**

(For cases/controls who reported eating outside the home, the number who are known to have consumed chicken when eating out, known not to have consumed chicken when eating out and the number for which information is not available on the foods eaten outside the home)

Table 21: Consumption of chicken eaten outside the home, combining those who ate outside the home but did not eat chicken and those who reported no eating outside the home.

Ate chicken outside the home	Case (%)	Control (%)
No (chicken outside home or eating outside home)	475 (60.2)	1152 (72.5)
Yes (chicken eaten out)	177 (22.4)	210 (13.2)
Missing/not specified	137 (17.4)	227 (14.3)
Total	**789**	**1589**

(The number of cases/controls who are known to have ate chicken outside the home, compared to the number known not to have eaten out or to have eaten out but not consumed eaten when eating out)

4.11 Consumption of foods prepared at home

The questionnaire collected information about consumption of a number foods prepared at home in the five days before onset or completing the questionnaire.

4.11.1 Eating chicken prepared at home.

61.1% (482) of cases and 67.7% (1076) of controls reported eating chicken prepared at home, this was not significantly different (odds ratio 0.89 (0.73 to 1.09), p = 0.264) (Table 22), and was not significant in the adjusted odds ratio 0.941 (0.768-1.152) (Table 44).

4.11.2 Eating red meat prepared at home

46.1% (364) of cases and 62.6% (995) of controls reported eating red meat prepared at home, this was a significant difference (odds ratio = 0.60 (0.49 to 0.72), p < 0.001) (Table 23). These results indicate that eating red meat prepared at home was associated with a reduced risk of *Campylobacter* infection.

4.11.3 Consumption of cooked meat at home

47.0% (371) of cases and 63.3% (1006) of controls reported eating cooked meat at home, this was a significant difference (odds ratio = 0.58 (0.48 to

0.71), p < 0.001) (Table 24). These results indicate that eating cooked meat at home was associated with a reduced risk of *Campylobacter* infection.

4.11.4 Consumption of salads or raw vegetables at home

51.3% (405) of cases and 65.8% (1046) of controls reported eating salads or raw vegetables at home, this was a significant difference (odds ratio = 0.62 (0.51 to 0.75), p < 0.001) (Table 25). These results indicate that eating salads or raw vegetables at home was associated with a reduced risk of *Campylobacter* infection.

This protective effect was also observed when considering food which was eaten at home and had been washed with tap water. The odds ratio for salads washed with tap water was 0.65 (0.54 to 0.79) and for raw vegetables washed with tap water was 0.50 (0.41 to 0.60) (Tables 30 & 31).

4.11.5 Consumption of shellfish at home

5.4% (43) of cases and 7.0% (112) of controls reported eating shellfish prepared at home, this was not a significant (odds ratio = 0.81 (0.55 to 1.18), p = 0.246) (Appendix Table 25).

5.11.6 Consumption of pre-packed sandwiches

12.5% (99) of cases and 17.2% (273) of controls reported eating pre-packed sandwiches, this was a significant difference (odds ratio = 0.75 (0.58 to 0.97), p = 0.0249) (Table 26). Whilst these results indicate that eating pre-packed sandwiches was associated with a reduced risk of *Campylobacter* infection, it must be taken into consideration that the upper end of the conference interval (0.97) is close to 1 and the strength of the association was not as strong as for some of the other variables that appeared to be protective, including salads and raw vegetables prepared at home. It should also be borne in mind that a response to the question was missing from questionnaires for 14.7% cases and 10.0% controls. Additionally no information was collected regarding the type of sandwiches consumed.

4.11.7 Consumption of raw/unpasteurised milk

4.4% (35) of cases and 8.8% (140) of controls reported drinking raw/unpasteurised milk, this was a significant difference (odds ratio = 0.50 (0.34 to 0.75), p = 0.00037) (Table 27). However, it is likely that a number of participants misunderstood this question and the meaning of the description of raw/unpasteurised milk. From the total of 175 participants who responded ‘Yes’ to this question 140 provided details of where the milk was purchased. The majority of these, named supermarkets (who are not permitted to sell unpasteurised milk) or other store chains as the source of milk, with only 12 participants reporting a location that was coded as ‘other’ in the database. Therefore it is likely that this data does not accurately represent the drinking of raw/unpasteurised milk.

4.11.8 Attending barbecues or picnics

8.7% (69) of cases and 6.2% (98) of controls reported attending a barbecue or picnic in the five days before onset or completing the questionnaire, this

was a significant difference (odds ratio = 1.47 (1.05 to 2.05), p = 0.0178) (Table 28).

4.11.9 Consumption of any pre-packed ready to eat foods

26.6% (210) of cases and 38.6% (614) of controls reported consumption of any pre-packed ready to eat foods, this was a significant difference (odds ratio 0.60 (0.49 to 0.72), p < 0.001) (Table 29). These results indicate that eating pre-packed ready to eat foods was associated with a reduced risk of infection. Of the 824 participants who responded 'Yes' to this question, 216 provided details of the type of food consumed. There was no commonly identified food and a wide range of food types was reported, ranging from biscuits, yoghurts, sandwiches, pasta, chicken products, sausages, etc, and multiple food types were reported by some participants. Therefore it is not possible to draw any firm conclusions about the risks associated with specific type of pre-packed ready to eat foods and *Campylobacter* infection.

Table 22: Consumption of chicken prepared at home

Chicken at home	**Case (%)**	**Control (%)**
No	211 (26.7)	421 (26.5)
Yes	482 (61.1)	1076 (67.7)
Not sure	45 (5.7)	4 (0.3)
Missing	51 (6.5)	88 (5.5)
Total	**789**	**1589**

Odds ratio 0.89 (0.73 to 1.09), p = 0.264

Table 23: Consumption of red meat prepared at home

Red Meat at home	**Case (%)**	**Control (%)**
No	294 (37.3)	480 (30.2)
Yes	364 (46.1)	995 (62.6)
Not sure	45 (5.7)	8 (0.5)
Missing	86 (10.9)	106 (6.7)
Total	**789**	**1589**

Odds ratio = 0.60 (0.49 to 0.72), p < 0.001

Table 24: Consumption of cooked meat, eg ham or salami at home

Cooked meat at home	**Case (%)**	**Control (%)**
No	298 (37.8)	471 (29.6)
Yes	371 (47.0)	1006 (63.3)
Not sure	34 (4.3)	6 (0.4)
Missing	86 (10.9)	103 (6.5)
Total	**789**	**1589**

Odds ratio = 0.58 (0.48 to 0.71), p < 0.001

Table 25: Consumption of salads or raw vegetables (including coleslaw) at home

Salads/raw vegetables at home	Case (%)	Control (%)
No	279 (35.4)	448 (28.2)
Yes	405 (51.3)	1046 (65.8)
Not sure	30 (3.8)	5 (0.3)
Missing	75 (9.5)	90 (5.7)
Total	**789**	**1589**

Odds ratio = 0.62 (0.51 to 0.75), $p < 0.001$.

Table 26: Consumption of pre-packed sandwiches

Pre-packed sandwiches	Case (%)	Control (%)
No	558 (70.7)	1155 (72.7)
Yes	99 (12.5)	273 (17.2)
Not sure	16 (2.0)	2 (0.1)
Missing	116 (14.7)	159 (10.0)
Total	**789**	**1589**

Odds ratio = 0.75 (0.58 to 0.97), $p = 0.0249$

Table 27: Consumption of raw/unpasteurised milk (in drink or with cereal)

Raw/unpasteurised milk	Case (%)	Control (%)
No	634 (80.4)	1280 (80.6)
Yes	35 (4.4)	140 (8.8)
Not sure	5 (0.6)	0
Missing	115 (14.6)	169 (10.6)
Total	**789**	**1589**

Odds ratio = 0.50 (0.34 to 0.75), $p = 0.00037$.

Table 28: Attending barbecues or picnics

Barbecues or picnics	Case (%)	Control (%)
No	706 (89.5)	1475 (92.8)
Yes	69 (8.7)	98 (6.2)
Missing	14 (1.8)	16 (1.0)
Total	**789**	**1589**

Odds ratio = 1.47 (1.05 to 2.05), $p = 0.0178$.

Table 29: Consumption of any pre-packed ready to eat foods.

Pre-packed ready to eat foods	Case (%)	Control (%)
No	541 (68.6)	944 (59.4)
Yes	210 (26.6)	614 (38.6)
Missing	38 (4.8)	31 (1.9)
Total	**789**	**1589**

Odds ratio 0.60 (0.49 to 0.72), $p < 0.001$.

4.12 Drinking bottled water

Information was collected about drinking bottled water in the five days before onset or completing the questionnaire.

51.1% (403) of cases and 49.9% (793) of controls reported drinking bottled water, in the unadjusted odds ratio this was not significantly different (odds ratio = 1.18 (0.98 to 1.41), p = 0.067) (Appendix Table 26). But in the adjusted odds ratio when age/sex were taken into account this was identified as a slight risk factor for *Campylobacter* infection 1.288 (1.068- 1.552) (Table 44).

Among those who reported drinking bottled water, cases were significantly more likely than controls to have drank still bottled water, but there was no significant difference for the drinking of sparkling bottled water (Appendix Tables 27& 28).

Participants were also asked to provide information on the number of glasses drank. In this context a glass was about a third of a pint or about 200 ml.

From the 363 cases and 681 controls that reported drinking still bottled water, 84.0% (305) and 93.2% (635) respectively provided information on the number of glasses drank, however many provided an estimate of the number of glasses drank and some reported bottles drank. Therefore it was impossible to conduct any meaningful analysis with this information.

4.13 Drinking water from other sources

Questions were asked about drinking water from other sources, namely a drinks dispenser, drinking fountain or from a river/stream. There was no significant difference between cases and controls in the consumption of water from any of these three sources (Appendix Tables 29 to 31).

4.14 Consumption of fresh vegetables, salads and fruit washed in tap water at home

Information was collected about eating raw vegetables, salads and fruit washed in tap water at home in the five days before onset or completing the questionnaire.

26.4% (209) of cases and 44.1% (701) of controls reported eating raw vegetables washed in tap water at home, this was a significant difference (odds ratio = 0.50 (0.41 to 0.60), $p < 0.001$) (Table 30).

38.0% (300) of cases and 50.7% (806) of controls reported eating salads washed in tap water at home, this was a significant difference (odds ratio 0.65 (0.54 to 0.79), p < 0.001) (Table 31).

42.6% (336) of cases and 66.8% (1058) of controls reported eating fruit washed in tap water at home, this was a significant difference (odds ratio = 0.41 (0.34 to 0.49), p < 0.001) (Table 32).

These results indicate that eating raw vegetables, salads or fruit washed in tap water at home were all associated with a significantly reduced risk of *Campylobacter* infection.

Table 30: Consumption of raw vegetables washed in tap water at home

Raw vegetables washed in tap water	**Case (%)**	**Control (%)**
No	473 (59.9)	788 (49.6)
Yes	209 (26.5)	701 (44.1)
Not sure	46 (5.8)	12 (0.8)
Missing	61 (7.7)	88 (5.5)
Total	**789**	**1589**

Odds ratio = 0.50 (0.41 to 0.60), p < 0.001

Table 31: Consumption of salads washed in tap water at home

Salads washed in tap water	**Case (%)**	**Control (%)**
No	398 (50.4)	698 (43.9)
Yes	300 (38.0)	806 (50.7)
Not sure	50 (6.3)	11 (0.7)
Missing	41 (5.2)	74 (4.7)
Total	**789**	**1589**

Odds ratio 0.65 (0.54 to 0.79), p < 0.001

Table 32: Consumption of fruit washed in tap water at home

Fruit washed in tap water	**Case (%)**	**Control (%)**
No	367 (46.5)	473 (29.8)
Yes	336 (42.6)	1058 (66.6)
Not sure	44 (5.6)	11 (0.7)
Missing	42 (5.3)	47 (3.0)
Total	**789**	**1589**

Odds ratio = 0.41 (0.34 to 0.49), p < 0.001

4.15 Water supply

4.15.1 Validation of household water source

The information regarding sources of water supply provided by participants in the study questionnaire (Table 33) were validated using the lists of private water supplies supplied by Aberdeenshire Environmental Health and Aberdeen City Environmental Health and the observations made by the water testing team during their visits for collection of the water samples (for those who participated in the water testing component of the study). As a result of these observations, a decision was made to create a derived field containing information regarding the final water source assigned to each participant to be used in the analysis (Table 34).

Appendix Table 32 provides details of the 61 participants for whom there was a lack of agreement between the information on the questionnaire, details in the private water supply database provided by the Environmental Health Departments and the observations made by the water testing team, and the justification for the final water source assigned to the participant. This 61 included participants who reported being 'not sure' of their water supply or for whom the answer was missing. In many instances using the information from the other sources, it was possible to determine the type of the water supply. All sources of information were not available for all participants, as not all participated in the water testing component and the data provided by the Environmental Health Teams covers only the private water supplies known to the local authority. Whilst these lists are regularly updated, the most up to date information may not always be available on all private water supplies. Similarly houses that were originally on a private water supply may have changed to a mains supply. Appendix Table 33 provides a comparison of the water sources from questionnaires and the final water source after validation.

Table 34 provides information on the final source of water supply assigned to participants. 8.0% (63) of cases and 2.8% (45) of controls had a private water supply. 91.6% (723) of cases and 96.6% (1541) of controls had a mains supply as their water source. Three controls and one case were believed to have both a private water supply and a mains supply. For two cases the water source was not established, even after using all the available sources of information.

Table 33: Answers from the questionnaire, as to source of household water supply.

Water source from questionnaire	Case (%)	Control (%)
Mains Water (Scottish Water)	703 (89.1)	1520 (95.7)
Private Water Supply (eg spring well)	61 (7.7)	46 (2.9)
Both	5 (0.6)	6 (0.4)
Unsure	13 (1.6)	13 (0.8)
Missing	7 (0.9)	4 (0.3)
Total	**789**	**1589**

Table 34: Final water source used for analysis.

Final water source	Case (%)	Control (%)	Total (%)
Mains Supply	723 (91.6)	1541 (97.0)	2264 (95.2)
Private Supply	63 (8.0)	45 (2.8)	108 (4.5)
Both	1 (0.1)	3 (0.2)	4 (0.2)
Not known	2 (0.3)	0	2 (0.1)
Total	**789**	**1589**	**2379**

4.15.2 Comparison of cases and controls on mains and private water supplies

For the following analysis participants with the final water supply coded as private water supply or a mains supply were selected.

When comparing mains and private water supplies between cases and controls, 8.0% (63) of cases had a private water supply, compared to 2.8% (45) of controls, this was a significant difference (odds ratio 2.98 (1.98 to 4.50) $p < 0.001$) (Table 35). This odds ratio of 2.98 is unadjusted and takes no account of age, sex or any of the other factors considered in this study. The adjusted odds ratios are present in Table 44, and show that after adjusting for these factors private water supplies are a significant risk factor for *Campylobacter* infection (when adjusted for age and sex only odds ratio 3.062 (2.056-4.562) (Table 44)

When considering adults on mains or private water supplies, 7.0% (48) of adult cases had a private water supply compared to 3.0% (40) of adult controls, this was a significant difference (odds ratio 2.46 (1.57 to 3.87) $p < 0.001$) (Appendix Table 34)

When considering children on mains or private water supplies, 14.4% (15) of child cases had a private water supply compared to 2.1% (5) of child controls, this was a significant difference (odds ratio 8.09 (2.65 to 26.32) $p < 0.001$) (Appendix Table 35), however it should be noted that the numbers of children (cases and controls) on a private water supply are relatively small, as evident in the wide confidence interval for the odds ratio.

The unadjusted odds ratio of having a private water supply for child cases was 8.09 (2.65 to 26.32) compared to 2.46 (1.57 to 3.87) for adult cases. A private water supply was reported for 14.4% of child cases compared to 7.0% of adult cases. Again consideration should be given to the fact that the number of child cases is relatively small.

It was interesting to note, that for cases in the 1-4 years age group 76.1% (35) had a mains supply a 23.9% (11) had a private water supply, compared to controls of the same age band, where 98.8% (84) had a mains supply and only 1.2% (1) a private water supply (Appendix Table 36).

4.15.3 Association between water source and month of study

(Study month was defined as the month the questionnaire was received at HPS).

The study was coded to season of the year, as per definition used by the Met Office, with December, January and February considered as winter (Appendix Table 37).

When considering the effect of season on private water supply, and comparing the seasonal pattern of risk for people with private water supplies against the rest, logistic regression adjusted for age and sex, showed there was no significant season by private water supply interaction, ($X^2 = 0.903$, df = 3, $p = 0.825$).

4.15.4 Association between water source and local authority area

107 of the 108 private water supplies reported were from participants resident in Aberdeenshire, with only one private water supply reported from Aberdeen City (Appendix Table 38).

When considering the risk of *Campylobacter* infection associated with private water supplies for the population of Aberdeenshire 13.7% (62) of cases had a private water supply compared to 5.7% (45) of controls (odds ratio 2.63 (1.72 to 4.01), $p < 0.001$).

4.15.5 Interaction between water source and overnight stay outside the study area

When considering the interaction between private water supplies and an overnight stay outside the study area, the estimates (not shown) demonstrated that the effect of private water supplies is more of a risk factor for *Campylobacter* infection when there is no history of an overnight stay outside the study area compared to those with a travel history. Table 44 shows this effect modification in that the odds ratio for private water supply increases to 3.3, from 3.1, when adjusting for an overnight stay outside the area.

However when Bonferroni correction is applied to take account of multiple testing, this is no longer significant.

4.15.6 Interaction between water source and contact with farm animals

There was no significant interaction in the adjusted odds ratio between having contact with a farm animal and private water supplies (Table 44).

4.15.7 Interaction between water source and drinking bottled water

When considering the interaction between private water supplies and drinking bottle water, the estimates (not shown) demonstrated that the effect of private water supplies is more of a risk factor for *Campylobacter* infection when there is no history of drinking bottled water compared to those drinking bottled water. Table 44 shows this effect modification in that the odds ratio for private water supply increases to 3.3, from 3.1, when adjusting for drinking bottled water.

However when Bonferroni correction is applied to take account of multiple testing, this is no longer significant.

4.15.8 Interaction between water source and taking part in water activities

When considering the interaction between private water supplies and taking part in water activities, the estimates (not shown) demonstrated an effect. However when Bonferroni correction is applied to take account of multiple testing, this is no longer significant. Furthermore the number of cases and controls participating in any water activity is very small.

4.15.9 Association between water source and other members in the household with similar symptoms

641 cases reported that others lived in the same household, 90.9% (583) had a mains supply and 8.7% (56) a private water supply, for one participant the water source was unknown and for one was reported as both a mains and private water supply.

12.7% (74) of cases on a mains supply reported someone else in the same household also had similar symptoms, compared to 23.2% (13) of those on a private water supply. Cases on a private water supply were significantly more likely to report that someone else in the same household had similar symptoms than those on a mains supply (chi square = 4.81, p = 0.0283) (Table 37).

4.15.10 Association between water source and changes in the water supply

Questions were asked about any changes in the water supply in the five days before onset or completing the questionnaire.

The number of participants reporting that their water supply had an unpleasant taste, smell or was dirty was small, making the drawing of any conclusions difficult. Interestingly, 9.5% (6) of cases on private water supply reported the water being dirty compared to 2.2% (1) control on a private water

supply and 2.3% (17) of cases and 1.3% (20) of controls on a mains supply (Appendix Tables 40 to 42).

4.16 Description of Private Water Supplies

For those participants on a private water supply the questionnaire collected additional information about any treatment of the supply.

Among cases on a private water supply 23.8% (15) reported the supply was treated and 53.9% (34) that the supply was not treated. Among controls on a private water supply 31.1% (14) reported the supply was treated and 44.4% (20) that the supply was not treated. For 22% (14) of cases and 24.4% (11) of controls information was either not recorded or reported as 'not sure' (Table 38).

When comparing those participants with a private water supply who provided a 'yes/no' response regarding the treatment of the water supply, there was no significant difference in the proportion of treated private water supplies among cases and controls (chi-square 0.99, p = 0.321).

Information on the type of treatment system used is provided in Appendix Tables 43 to 45. More than one type of treatment system was reported by some cases and controls. Four cases who reported having a UV filter also reported another treatment system as well: with a mineral filter, rope filter, RO filter and a pH filter reported. Three controls reported having both a UV filter and the supply been chlorinated, one control reported having both a UV filter and coare particle filter and another control reported having both a UV filter and a pH filter.

Table 35: Cases and controls on mains or private water supplies

Water source	**Case (%)**	**Control (%)**
Mains	723 (92.0)	1541 (97.2)
PWS	63 (8.0)	45 (2.8)
Total	**786**	**1586**

Odds ratio 2.98 (1.98 to 4.50) p < 0.001

Figure 13: Number of cases and controls with a private water supply by month the questionnaire was received.

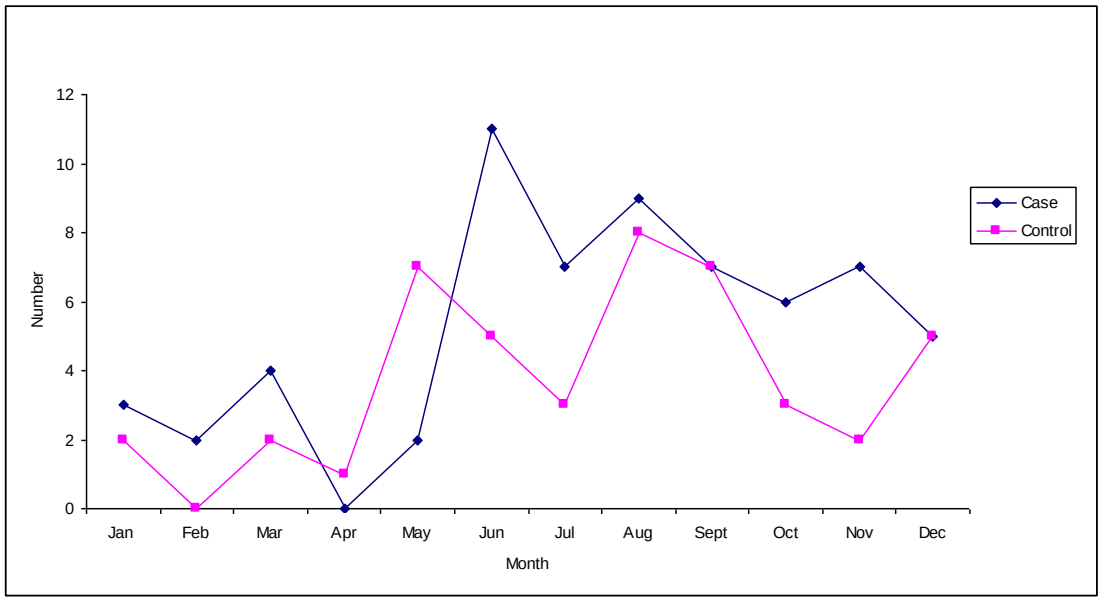


Table 36: Water source for those with no overnight stay outside the study area in the 14 days before onset or completing the questionnaire.

Water source	Case (%)	Control (%)
Mains	461 (90.4)	1222 (97.3)
PWS	49 (9.6)	34 (2.7)
Total	**510**	**1256**

Table 37: Number of cases on mains and private water supplies who reported other members of the same household to be ill with similar symptoms

Selected for cases who report that others lived in the same household.

Number of others in the household with similar symptoms	Final water source	
	Mains (%)	PWS (%)
0	509 (87.3)	43 (76.7)
1	57 (9.8)	11 (19.6)
2	11 (1.9)	1 (1.8)
3	6 (1.0)	1 (1.8)
Total	**583**	**56**

Table 38: Treatment of private water supply

(Selected for participants where the final water source was a private water supply)

Treated	Case (%)	Control (%)
No	34 (75.6)	20 (44.4)
Yes	15 (33.3)	14 (31.1)
Not Sure	8 (17.8)	7 (15.6)
Not recorded	6 (13.3)	4 (8.9)
Total	**63**	**45**

4.17 Amount of tap water consumed by participants

Information was collected about the amount of water consumed, either on its own or in juice or squash, in their own home, at work/school and elsewhere.

Participants were asked to estimate their approximate consumption of tap water in these categories: ‘no’, ‘not sure’, ‘1-3 glasses’, ‘4-6 glasses’ or ‘7+ glasses’ a day.

Among cases who reported drinking tap water at home 54.2% (428) drank 1 or more glasses and 30.3% (239) drank no tap water on its own. Among controls 66.2% (1052) drank 1 or more glasses and 25.8% (410) drank no tap water on its own, this was a significant difference (odds ratio 0.70 (0.57 to 0.85), p = 0.0003). Whilst the analysis indicated cases were less likely to drink tap water on its own at home, the percentage of missing answers was also significantly greater among cases 13.8% compared to 7.4% for controls (chi-square = 24.92, p < 0.001) and therefore this finding should be viewed with caution (Table 39) additionally the size of glasses considered is likely to be variable.

There was no significant difference between cases and controls on a private water supply in whether they reported drinking tap water on its own at home (Appendix Table 46).

There was no significant difference between cases on a private water supply and those with a mains supply in reporting drinking tap water on its own at home (Appendix Table 46).

There was no significant difference between cases and controls in the reporting of drinking tap water in juice or squash at home (Appendix Table 47 & 48).

There was no significant difference between cases and controls in reporting drinking water either on its own or in juice at work/ school or elsewhere (Appendix Tables 49 to 52)

Table 39: Number of participants drinking tap water on its own at home

Number of glasses	Case (%)	Control (%)
No	239 (30.3)	410 (25.8)
Not sure	13 (1.6)	9 (0.6)
1-3	320 (40.6)	846 (53.2)
4-6	78 (9.8)	161 (10.1)
7 +	30 (3.8)	45 (2.8)
Missing	109 (13.8)	118 (7.4)
Total	**789**	**1589**

4.18 Recreational water activities

Information was collected about participation in recreational water activities, in the five days before onset or the five days prior to completing the questionnaire.

There were no significant differences between cases and controls in reporting taking part in swimming, canoeing, sailing, fishing or surfing. Taking part in diving in the sea was significantly more common among cases than controls (odds ratio 4.14 (1.84 to 9.53), $p < 0.001$), it must be taken into consideration that the number of cases reporting this exposure (2.5%) was very small, and the confidence intervals are wide. Due to the small numbers, water activities were combined into a single variable for the calculation of adjusted odds ratio, which showed no significant association, adjusted odds ratio 1.107 (0.868-1.412) (Table 44).

4.19 Summary of unadjusted odds ratio

Table 40: Summary of unadjusted odds ratio for cases and well controls: Whole study area

Factor	Cases		Well controls		Odds ratio	P value
	Yes	Total Yes/no response	Yes	Total Yes/no response		
Antacids (adults)	56	649	98	1309	1.17 (0.82 to 1.67)	0.376
Antibiotics (adults)	45	646	78	1305	1.18 (0.79 to 1.75)	0.397
Omeprazole etc (adults)	126	669	116	1314	2.40 (1.81 to 3.17)	<0.001
Overnight stay outside area	273	785	330	1588	2.03 (1.67 to 2.47)	<0.001
Pets at home	399	785	724	1583	1.23 (1.03 to 1.46)	0.019
Farm animals	75	646	99	1232	1.50 (1.08 to 2.09)	0.011
PWS (PWS or Mains)	63	786	45	1586	2.98 (1.98 to 4.50)	<0.001
Bottled water	403	736	793	1565	1.18 (0.98 to 1.41)	0.067
Drinks dispenser	164	741	382	1541	0.86 (0.70 to 1.07)	0.163
Drinking fountain	49	701	124	1451	0.80 (0.56 to 1.15)	0.214
Drinking rivers etc	8	705	19	1445	0.86 (0.34 to 2.09)	0.725
Raw veg washed in tap water	209	682	701	1489	0.50 (0.41 to 0.61)	< 0.001
Salads washed in tap water	300	698	806	1504	0.65 (0.54 to 0.79)	< 0.001
Fruit washed in tap water	336	703	1058	1531	0.41 (0.34 to 0.49)	< 0.001
Eating outside the home	468	765	741	1573	1.77 (1.48 to 2.12)	< 0.001
Chicken at home	482	693	1076	1497	0.89 (0.73 to 1.09)	0.264
Red meat at home	364	658	995	1475	0.60 (0.49 to 0.72)	<0.001
Cooked meat at home	371	669	1006	1477	0.58 (0.48 to 0.70)	<0.001
Salads/raw veg at home	405	684	1046	1494	0.62 (0.51 to 0.75)	<0.001
Shellfish at home	43	653	112	1393	0.81 (0.55 to 1.18)	0.246
Pre-packed sandwiches	99	657	273	1428	0.75 (0.58 to 0.97)	0.0249
Barbecues & picnics	69	775	98	1573	1.47 (1.05 to 2.05)	0.0178
Pre-packed ready to eat foods	210	751	614	1558	0.60 (0.49 to 0.72)	<0.001
Swimming	121	766	243	1569	1.02 (0.80 to 1.31)	0.847
Canoeing	4	745	3	1514	2.72 (0.52 to 15.28)	0.173
Sailing	7	745	11	1516	1.30 (0.45 to 3.62)	0.591
Fishing	9	749	14	1520	1.31 (0.52 to 3.23)	0.531
Surfing	0	745	2	1514		
Diving in sea	20	747	10	1516	4.14 (1.84 to 9.53)	<0.001
Head under water	104	538	203	987	0.93 (0.70 to 1.21)	0.565

Table 41: Summary of unadjusted odds ratio for cases and ALL controls: Whole study area

Factor	Cases		All controls		Odds ratio	P value
	Yes	Total Yes/no response	Yes	Total Yes/no response		
Antacids (adults)	56	649	140	1575	0.97 (0.69 to 1.35)	0.844
Antibiotics (adults)	45	646	103	1567	1.06 (0.73 to 1.55)	0.736
Omeprazole etc (adults)	126	669	163	1585	2.02 (1.56 to 2.63)	<0.001
Overnight stay outside area	273	785	406	1894	1.95 (1.62 to 2.36)	<0.001
Pets at home	399	785	879	1890	1.19 (1.00 to 1.41)	0.0417
Farm animals	75	646	117	1477	1.53 (1.11 to 2.10)	0.0064
PWS (PWS or Mains)	63	786	60	1894	2.66 (1.82 to 3.89)	<0.001
Bottled water	403	736	963	1873	1.14 (0.96 to 1.36)	0.124
Drinks dispenser	164	741	466	1841	0.84 (0.68 to 1.03)	0.089
Drinking fountain	49	701	143	1739	0.84 (0.59 to 1.19)	0.306
Drinking rivers etc	8	705	26	1730	0.75 (0.31 to 1.75)	0.480
Raw veg washed in tap water	209	682	828	1781	0.51 (0.42 to 0.62)	<0.001
Salads washed in tap water	300	698	947	1798	0.68 (0.57 to 0.81)	<0.001
Fruit washed in tap water	336	703	1245	1835	0.43 (0.36 to 0.52)	<0.001
Eating outside the home	468	765	894	1879	1.74 (1.46 to 2.07)	<0.001
Chicken at home	482	693	1279	1791	0.91 (0.75 to 1.11)	0.360
Red meat at home	364	658	1169	1767	0.63 (0.53 to 0.76)	<0.001
Cooked meat at home	371	669	1198	1768	0.59 (0.49 to 0.71)	<0.001
Salads/raw veg at home	405	684	1250	1787	0.62 (0.52 to 0.75)	<0.001
Shellfish at home	43	653	132	1671	0.82 (0.57 to 1.19)	0.280
Pre-packed sandwiches	99	657	332	1709	0.74 (0.57 to 0.95)	0.0139
Barbecues & picnics	69	775	116	1882	1.49 (1.08 to 2.05)	0.0116
Pre-packed ready to eat foods	210	751	744	1867	0.59 (0.49 to 0.71)	<0.001
Swimming	121	766	270	1870	1.11 (0.87 to 1.41)	0.373
Canoeing	4	745	3	1806	3.24 (0.62 to 18.23)	0.204
Sailing	7	745	13	1809	1.31 (0.47 to 3.52)	0.567
Fishing	9	749	18	1813	1.21 (0.50 to 2.86)	0.638
Surfing	0	745	2	1806		
Diving in sea	20	747	11	1808	4.49 (2.04 to 10.05)	<0.001
Head under water	104	538	224	1184	1.03 (0.79 to 1.34)	0.840

Table 42: Summary of unadjusted odds ratio for cases and WELL controls: Aberdeenshire only

Factor	Cases		Well controls		Odds ratio	P value
	Yes	Total Yes/no response	Yes	Total Yes/no response		
Antacids (adults)	30	362	56	656	0.97 (0.59 to 1.57)	0.891
Antibiotics (adults)	26	361	40	658	1.20 (0.70 to 2.06)	0.485
Omeprazole etc (adults)	76	373	50	661	3.13 (2.10 to 4.67)	<0.001
Overnight stay outside area	140	455	166	794	1.68 (1.28 to 2.20)	<0.001
Pets at home	265	454	408	790	1.31 (1.03 to 1.67)	0.0219
Farm animals	66	387	78	645	1.49 (1.03 to 2.16)	0.0259
PWS (PWS or Mains)	62	454	45	792	2.63 (1.72 to 4.01)	<0.001
Bottled water	221	425	408	783	1.00 (0.78 to 1.27)	0.971
Drinks dispenser	78	428	163	778	0.84 (0.62 to 1.15)	0.257
Drinking fountain	29	406	51	731	1.03 (0.62 to 1.69)	0.916
Drinking rivers etc	7	412	13	732	0.96 (0.34 to 2.59)	0.924
Raw veg washed in tap water	121	392	360	749	0.48 (0.37 to 0.63)	<0.001
Salads washed in tap water	184	404	413	753	0.69 (0.54 to 0.88)	0.0025
Fruit washed in tap water	197	398	527	765	0.44 (0.34 to 0.57)	<0.001
Eating outside the home	276	447	348	785	2.03 (1.59 to 2.59)	<0.001
Chicken at home	269	410	528	747	0.79 (0.61 to 1.03)	0.0764
Red meat at home	219	387	535	746	0.51 (0.39 to 0.67)	<0.001
Cooked meat at home	211	382	509	741	0.56 (0.43 to 0.73)	<0.001
Salads/raw veg at home	233	396	525	752	0.62 (0.48 to 0.80)	0.0001
Shellfish at home	25	382	59	694	0.75 (0.45 to 1.25)	0.252
Pre-packed sandwiches	52	379	127	715	0.74 (0.51 to 1.06)	0.0855
Barbecues & picnics	43	449	54	787	1.44 (0.93 to 2.23)	0.0877
Pre-packed ready to eat foods	105	432	282	778	0.56 (0.43 to 0.74)	<0.001
Swimming	67	444	121	784	0.97 (0.70 to 1.36)	0.872
Canoeing	3	430	2	764	2.68 (0.36 to 22.91)	0.263
Sailing	5	430	9	766	0.99 (0.29 to 3.25)	0.985
Fishing	5	433	6	768	1.48 (0.39 to 5.51)	0.514
Surfing	0	430	2	764	0 (0 to 7.22)	0.288
Diving in sea	8	430	5	765	2.88 (0.85 to 10.18)	0.0535
Head under water	62	307	105	488	0.92 (0.64 to 1.33)	0.656

Table 43: Summary of unadjusted odds ratio for cases and All controls: Aberdeenshire only

Factor	Cases		All controls		Odds ratio	P value
	Yes	Total Yes/no response	Yes	Total Yes/no response		
Antacids (adults)	30	362	73	778	0.87 (0.55 to 1.39)	0.548
Antibiotics (adults)	26	361	47	779	1.21 (0.71 to 2.04)	0.453
Omeprazole etc (adults)	76	373	65	784	2.83 (1.95 to 4.11)	<0.001
Overnight stay outside area	140	455	199	938	1.65 (1.27 to 2.14)	<0.001
Pets at home	265	454	496	935	1.24 (0.98 to 1.57)	0.0616
Farm animals	66	387	93	768	1.49 (1.04 to 2.13)	0.0213
PWS (PWS or Mains)	62	454	60	937	2.31 (1.56 to 3.42)	<0.001
Bottled water	221	425	499	929	0.93 (0.74 to 1.18)	0.557
Drinks dispenser	78	428	192	922	0.85 (0.63 to 1.15)	0.266
Drinking fountain	29	406	58	872	1.08 (0.66 to 1.75)	0.745
Drinking rivers etc	7	412	18	873	0.82 (0.31 to 2.10)	0.660
Raw veg washed in tap water	121	392	424	886	0.49 (0.38 to 0.63)	<0.001
Salads washed in tap water	184	404	480	892	0.72 (0.56 to 0.92)	0.0058
Fruit washed in tap water	197	398	618	908	0.46 (0.36 to 0.59)	<0.001
Eating outside the home	276	447	415	929	2.00 (1.58 to 2.53)	<0.001
Chicken at home	269	410	627	887	0.79 (0.61 to 1.02)	0.0658
Red meat at home	219	387	625	885	0.54 (0.42 to 0.70)	<0.001
Cooked meat at home	211	382	603	878	0.56 (0.44 to 0.73)	<0.001
Salads/raw veg at home	233	396	624	890	0.61 (0.47 to 0.79)	<0.001
Shellfish at home	25	382	69	822	0.76 (0.46 to 1.26)	0.2655
Pre-packed sandwiches	52	379	151	843	0.73 (0.51 to 1.04)	0.0685
Barbecues & picnics	43	449	60	933	1.56 (1.00 to 2.36)	0.0370
Pre-packed ready to eat foods	105	432	341	924	0.55 (0.42 to 0.72)	<0.001
Swimming	67	444	132	927	1.07 (0.77 to 1.49)	0.675
Canoeing	3	430	2	903	3.17 (0.43 to 27.08)	0.194
Sailing	5	430	9	905	1.17 (0.34 to 3.84)	0.778
Fishing	5	433	9	907	1.17 (0.34 to 3.82)	0.784
Surfing	0	430	2	903	0 (0 to 8.53)	0.328
Diving in sea	8	430	5	904	3.41 (1.01 to 12.04)	0.023
Head under water	62	307	155	619	1.02 (0.71 to 1.46)	0.905

4.20 Adjusted odds ratios.

Table 44: Adjusted odds ratio for cases and well controls: Whole study area
Adjusted for age, sex and factor in table

Factor		95% CI			Chi Sq test of effect modification	Df	P value
		Estimate	Lower	Upper			
PWS		3.062	2.056	4.562			
PWS	No	1					
	Yes	3.257	2.174	3.257			
					3.863	2	0.145
Abroad	No	1					
	Yes	3.344	2.502	4.470			
	Not stated	0.577	0.263	1.268			
PWS	No	1					
	Yes	3.269	2.183	4.896			
					3.800	1	0.051
Abroad No & Not sure combined	No	1					
	Yes	3.378	2.528	4.514			
PWS	No	1					
	Yes	3.217	2.144	4.826			
					4.916	1	0.027
Overnight Stay	No	1					
	Yes	2.133	1.750	2.599			
PWS	No	1					
	Yes	2.765	1.831	4.178			
Farm Animals	No	1			1.547	2	0.461
	Yes	1.265	0.898	1.782			
	Not stated	0.797	0.637	0.996			
PWS	No	1					
	Yes	2.815	1.864	4.250			
					0.658	1	0.417
Farm Animals No & Not sure combined	No	1					
	Yes	1.320	0.939	1.855			
PWS	No	1					
	Yes	2.932	1.964	4.377			
Animals (pets) No & Not sure combined	No	1			0.739	1	0.390
	Yes	1.238	1.033	1.483			
PWS	No	1					
	Yes	3.038	2.026	4.557			
					0.304	1	0.582

Antacids	No	1					
	Yes	1.173	0.827	1.664			
	No	1					
PWS	Yes	2.957	1.976	4.425			
					0.045	1	0.831
	No	1					
Antibiotics	Yes	1.134	0.783	1.641			
PWS	No	1					
	Yes	3.165	2.112	4.744			
					0.236	1	0.627
Protein pump Inhibitors/ H_2-Receptor antagonists	No	1					
	Yes	2.732	2.052	3.639			
PWS	No	1					
	Yes	3.118	2.088	4.657			
Any Antibiotic/ antacid	No	1			1.020	1	0.312
	Yes	1.789	1.433	2.232			
	Unk/ Mis	1.714	0.779	3.775			
PWS	No	1					
	Yes	3.121	2.090	4.660	1.002	2	0.317
Any Antibiotic/ acid No & Not sure combined	No	1					
	Yes	1.781	1.427	2.222			
PWS	No	1					
	Yes	2.970	1.980	4.454			
Chicken prepared at home	No	1			4.535	3	0.209
	No answ	1.131	0.761	1.682			
	Not sure	22.505	7.924	63.916			
	Yes	0.937	0.765	1.147			
PWS	No	1					
	Yes	3.203	2.090	4.910			
					1.123	1	0.289
Chicken home Excluding note sure/missing	No	1					
	Yes	0.941	0.768	1.152			
PWS	No	1					
	Yes	3.299	2.194	4.960			
					3.407	2	0.182
Chicken Eaten out	No	1					
	Yes	2.118	1.677	2.676			
	Not state	1.134	0.840	1.531			
PWS	No	1					

	Yes	3.301	2.195	4.964			
Chicken	No	1			3.404	1	0.065
eaten out	Yes	2.083	1.655	2.622			
Not & not sure combined							
PWS	No	1					
	Yes	3.067	2.058	4.569			
Any	No	1			4.502	1	0.034
water	Yes	1.107	0.868	1.412			
Activity							
No / no answer combined							
PWS	No	1					
	Yes	3.271	2.187	3.271			
Bottled	No	1			4.551	2	0.103
Water	Not sure	9.840	4.999	9.840			
	Yes	1.288	1.069	1.288			
PWS	No	1					
	Yes	3.262	2.178	4.884			
					4.565	1	0.033
Bottled	No	1					
water	Yes	1.288	1.068	1.552			
PWS	No	1					
	Yes	2.793	1.856	4.201			
SIMD	Gp Q1	1			5.437	3	0.142
	Q2	1.466	1.101	1.952			
	Q3	1.944	1.409	2.680			
	Q4	1.581	1.155	2.164			
	Q5	1.368	1.010	1.853			

Potential confounding effects of known risk factors for *Campylobacter* were assessed through fitting logistic regression models adjusting for both age and sex. For each of the major factors identified in the study, e.g. travel etc, a logistic model was fitted including the effects of both private water supply and the factor. The adjusted odds ratios from these models are presented in Table 44. We then added in the interaction between private water supply and the factor and tested the significance of the interaction through a chi square test on the change in deviance, also presented in Table 44. If there is evidence of a significant interaction then this suggests that the effect of private water supply on the odds of having *Campylobacter* is modified by the other major risk factor.

The only factors that initially appear to show an interaction with private water supplies are overnight stay outside the study area, undertaking any water activity and drinking bottled water. However when the Bonferroni correction is

applied to take account of multiple testing, the p value considered significant would be 0.05/12 = 0.0042 (12 is the number of factors in which the interaction with private water supplies was considered), none of these three factors would then be considered to have a significant interaction with private water supplies. The use of a correction for multiple testing is justified as we have no prior hypotheses about the potential interactions from any one of the potential factors.

4.21 Associations between exposure factors and clinical symptoms

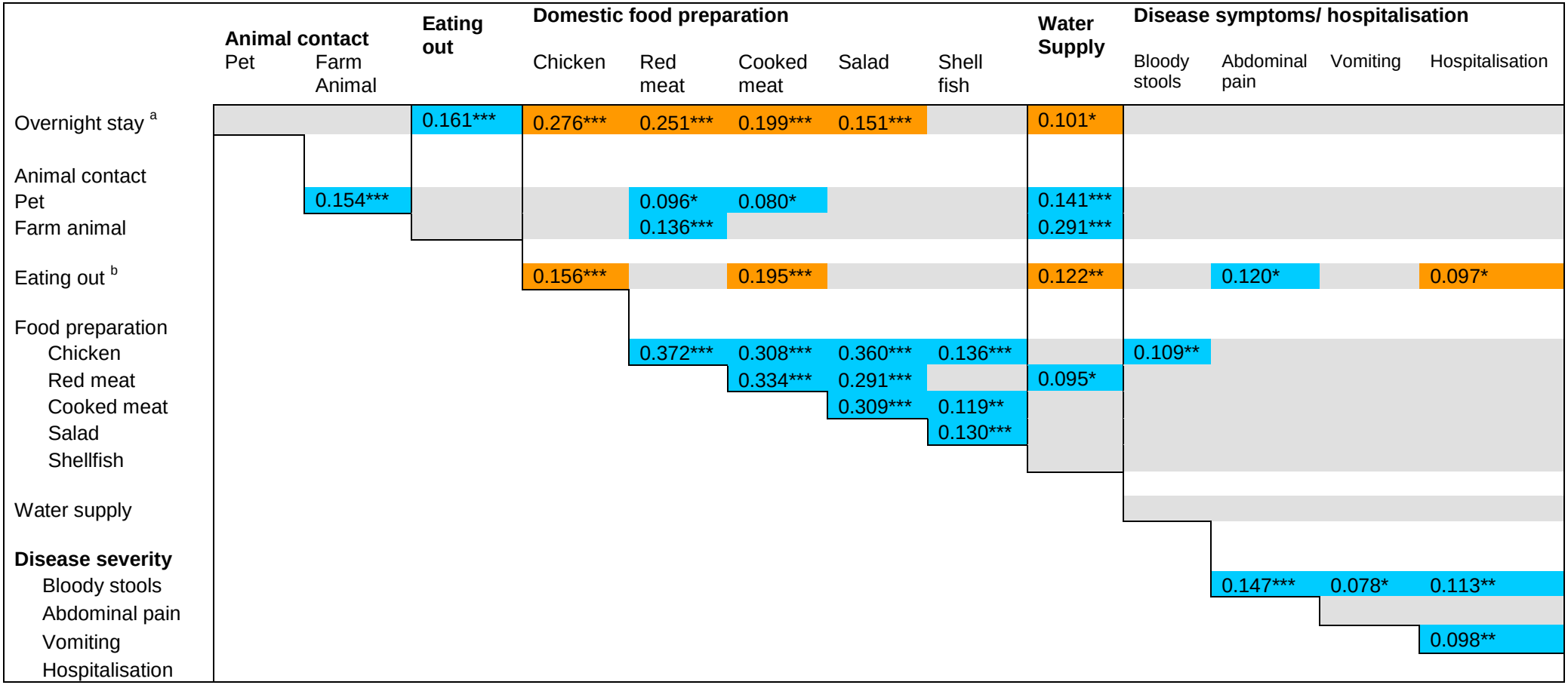
Pairwise statistical association for ten exposure factors and disease severity/hospitalisation were analysed, the result for each pair is shown in Table 45. The presence of association and whether it was positive or negative is indicated by a coloured cell, and the strength of the association is indicated by a value of the Cramer's V statistic and the categories p values. Some of the factors were certain to be associated e.g travel *versus* eating out, and their analysis merely confirmed strong expectations. Other factors had unknown association patterns e.g contact with farm animals *versus* having a private water supply and their analysis had important implications for subsequent results on associations between exposure characteristics and the host attributions of patients' *Campylobacter* strains as described in the MLST results chapter.

Two groups of factors were linked by a common activity or epidemiological origin, such that the factors in each group were likely to be associated. The first group contained five factors relating to food prepared at home (chicken, red meat, cooked meat, salad and shellfish) and these were positively and strongly associated in nine of the 10 possible combinations. The second group contained three clinical symptoms and hospitalisation, and these showed positive association for four of the six possible combinations.

Three further factors were expected to show some association; overnight stay outside the study area, eating out, and consumption of food prepared at home. Overnight stay outside the study area and eating out were positively associated. Overnight stay was negatively associated with eating four of the five foods groups prepared at home, and eating out was negatively associated with eating chicken and cooked meat prepared at home. The positive association between overnight stay outside the study area and eating out was due to both travel within the UK and abroad. The negative association between overnight stay outside the study area and eating food prepared at home were all due to an overnight stay abroad.

Further associations were identified among factors whose relationships were less easily predicted. Contact with pets, contact with farm animals and private water supply were all positively associated with each other. Private water supply was negatively associated with both an overnight stay outside the study area and eating out.

Table 45: Pairwise statistical associations between 10 infection risk exposures and four disease symptoms



Notes: Blue cells indicate significant positive association, orange cells indicate significant negative association and grey cells indicate no association. The cells indicating significant association give the Cramer's V value followed by the level of statistical significance expressed as * (0.05 > *P* > 0.01), ** (0.01 > *P* > 0.001) and *** (*P* 0.001).

[a,b] These variables consisted of three mutually-exclusive, yes-or-no response categories. The "overnight stay" categories were (i) no overnight stay, (ii) overnight stay elsewhere in the UK and Ireland, and (iii) overnight stay in an overseas country, and the "Eating out" categories were (i) no meal eaten out, (ii) meal eaten out but no chicken consumed, and (iii) meal eaten out and chicken consumed.

CHAPTER 5: Water testing results

During the pilot and the first year of the main study, all participants were invited to participate in the water testing component of the study. The study protocol was revised at the start of the second year of the study, so that only those on a private water supply were invited to participate in the water testing component.

The use of mains or private water supply reported in this section corresponds to the source coded for the analysis after the validation of the water source and therefore for a few participants differs from that reported on the questionnaire (Appendix Table 32).

The data for the water testing component includes all cases and controls and therefore is not limited to 'well' controls.

5.1.1 Consenting to participate in the water testing component

The overall rate of agreeing to participate in the water testing component of the study was 69%.

During the pilot 78% agreed to participate in the water testing, compared to 68% for the main study (Appendix Table 53). This apparent decline in the number of those consenting between the pilot and the main study, was a result of the change in the study protocol as described above.

Controls were significantly more likely to consent to participate in the water testing component than cases, 70% and 66% respectively (chi square = 6.194, df = 1, p = 0.013) (Table 46). Despite this difference, the participation rate for the water testing component of 66% by cases was still considered to be high.

Children, for whom the consent to participate in the water testing component would have been provided by the parent or guardian, had a rate of consenting, significantly higher than adults 76% and 68% respectively (chi square = 10.049, df = 1, p = 0.002.) (Table 47). When considering child case and controls consenting to the water testing component, 75% of child cases and 77% of child controls consented to the testing.

There was no significant difference in consenting to participate in the water testing for those resident in Aberdeen City or Aberdeenshire or for those resident in different deprivation categories (Appendix Table 54 and 55)

5.1.2 Water testing by participant

Although a total of 1855 participants agreed to participate in this component of the study, water samples were only obtained from 1006 participants. The majority of the difference resulted from those on a mains supply agreeing to take part in the water testing during the second year of the study, which had been restricted to those on private water supplies, as described above. Additionally, in some instances the water testing team were unable to make

contact with the participant to arrange a suitable time to visit and collect the sample, and in others no one was at home when a member of the water testing team visited.

87% (873) of samples tested were from the main study and 13% (133) from the pilot study.

71% (711) of samples tested were from controls and 29% (295) from cases (Table 48).

83% (836) of samples tested were from the households of adults participating in the study and 17% (170) form the households of children.

92% (925) of samples tested were from a mains supply, 8% (77) from a private water supply and four from a participant where the household water supply was coded as both. There were no samples from any supplies coded as not known (Appendix Table 56).

Table 46: Number of cases and controls consenting to participate in the water testing

Consenting to water testing	Case (%)	Control (%)
No	271 (34%)	561 (30%)
Yes	518 (66%)	1337 (70%)
Total	**789**	**1898**

Table 47: Number of children and adults consenting to participate in the water testing.

Consenting to water testing	Adult (%)	Child (%)
No	742 (32%)	90 (24%)
Yes	1567 (68%)	288 (76%)
Total	**2309**	**379**

Table 48: Number of cases and controls for whom water testing was conducted.

Water tested	Case (%)	Control (%)
Yes	295 (37%)	711 (38%)
No	494 (63%)	1187 (62%)
Total	**789**	**1898**

5.1.3 Number of days between onset and collection of water sample.

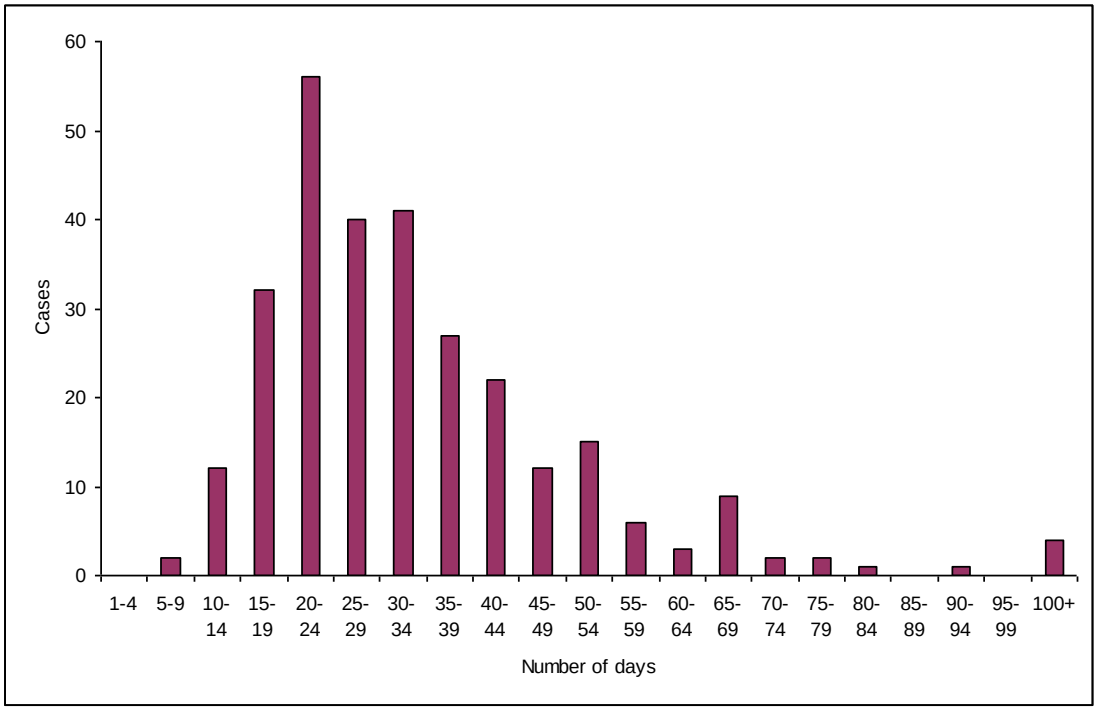
Data on the number of days between onset of illness and the collection of water sample was available for 287 cases.

The number of days between date of onset and the collection of the water sample being collected ranged from 6 to 153 days, a mean of 33.8 days (standard deviation 18.5 days) (Figure 14).

For adult cases the mean number of days between onset and the water sample being collected was 33.5 days (standard deviation 18.5 days: n=240). For child cases the mean was 35.7 days (standard deviation 19.1 days: n=47). There was no significant difference between adult and child cases in the time between onset and the water sample collection (t-test = 0.751, df = 285, p = 0.454).

For cases on a mains supply the mean number of days between onset and the water sample being collected was 33.3 days (standard deviation 17.4 days), for cases on a private water supply the mean was 36.9 days (standard deviation 24.4 days). There was no significant difference between cases on mains or private water supplies in the time between onset and water collection (t-test 1.149, d f= 285, p = 0.251).

Figure 14: Number of days between onset for cases and collection of the water sample.



5.2 Results of microbiological water testing

In the enumeration of coliforms, *E.coli* and Enterococci 200 cfu per ml was the maximum enumeration possible with the methodology. For such samples 200 was the value used in the t-test analysis, where applicable, although it is recognised for some of these samples this would not have been the true value.

5.2.1 Detection of coliforms

Coliforms were detected from a total of 65 water samples, 16 (24.6%) of these were from mains supplies and 48 (73.8%) from private water supplies.

Among mains supplies only 16 (1.7%) of the samples were positive for coliforms. Among private water supplies 48 (62%) were positive for coliforms (Table 49). Coliforms were significantly more likely to be detected from a private water supply than a mains supply (chi-square = 436.7, $p < 0.001$).

In 71% (12) of the mains supplies in which coliforms were detected, the enumeration detected 1-9 cfu per ml, (in 7 of which only 1 cfu per ml was detected). This low level of contamination was only detected in 21% (10) of the positive samples from private water supplies.

The highest level of coliform contamination in a mains supply was 145 cfu per ml detected from only one sample. In 14 of the samples from private water supplies coliforms were detected at a level of 200+ cfu per ml. These 14 samples account for 29% of private water supply samples that were positive for coliforms and 18% of all samples from private water supplies tested (Appendix Figure 1)

5.2.2 Detection of *E. coli*

E. coli was detected in a total of 26 samples, 25 of which were from private water supplies and one for which the validated water source was coded as both and in none from a mains supply (Table 50). *E. coli* was significantly more likely to be detected in samples from a private supply than in samples from a mains supply (chi-square = 308.01, $p < 0.001$).

In 52% (13) of the samples from private water supplies that tested positive for *E. coli*, the enumeration detected 1-9 cfu per ml. In 3% (2) the enumeration detected 200+ *E. coli* cfu per ml. (Appendix Figure 2)

E. coli O157 was not detected in any of the samples from either mains or private water supply.

5.2.3 Detection of Enterococci

Enterococci were significantly more likely to be detected in samples from private water supplies than mains supplies 32% (25) and 0.1% (1) respectively (chi-square 308.01, $p < 0.001$) (Table 51).

In this single positive sample from a mains supply, Enterococci were enumerated at 1 cfu per ml. Among the 25 positive samples from private

water supplies Enterococci, enumeration ranged from 1 to 150 cfu per ml (Appendix Figure 3).

5.2.4 Detection of *Campylobacter*

Campylobacter was only isolated from three water samples. Two of these were from private water supplies and the third sample was from a participant for whom the water source was coded as both private water supply and mains, with the water sample taken from the private water supply at the property (Table 52). The *Campylobacter* were detected after enrichment and therefore enumeration was not possible.

Table 49: Detection of coliforms in the water sample, by type of supply

Coliforms detected	**Mains (%)**	**PWS (%)**	**Both (%)**
Yes	16 (1.7)	48 (62)	1 (25)
No	909 (98)	29 (38)	3 (75)
Total	**925**	**77**	**4**

Table 50: Detection of *E. coli* in the water sample, by type of supply

***E.coli* detected**	**Mains (%)**	**PWS (%)**	**Both (%)**
Yes	0	25 (32)	1 (25)
No	925 (100)	52 (68)	3 (75)
Total	**925**	**77**	**4**

Table 51: Detection of Enterococci in water sample, by type of supply

Enterococci detected	**Mains (%)**	**PWS (%)**	**Both (%)**
Yes	1 (0.1)	25 (32)	1 (25)
No	924 (99.8)	52 (68)	3 (75)
Total	**925**	**77**	**4**

Table 52: Detection of *Campylobacter* in water sample, by type of supply

***Campylobacter* detected**	**Mains (%)**	**PWS (%)**	**Both (%)**
Yes	0	2 (2.6%)	1 (25%)
No	925 (100%)	75 (97.4%)	3 (75%)
Total	**925**	**77**	**4**

5.3 Differences in microbiological water quality between cases and controls on private water supplies

There was no significant difference between private water supplies belonging to cases and controls in detection of coliforms. Detected in supplies from 63% of cases and 62% of controls (chi-square 0.0009, df=1, p = 0.926) (Table 53).

There was no significant difference in the number of coliforms detected in positive private water supplies from cases and controls. The mean number of coliforms detected in positive samples from cases was 109 cfu per ml and from controls 73 cfu per ml (t-test = 1.150, df = 46, p = 0.138) (Appendix Figure 4).

E. coli was significantly more likely to be detected from a private water supply from a case than a control 42% and 21% respectively, however as the p value was 0.048 this had only just reached the level of statistical significance (chi-square = 3.919, df = 1, p = 0.048) (Table 54).

There was no significant difference in the number of *E. coli* detected in positive private water supplies from cases and controls. The mean number of *E. coli* detected in positive samples from cases was 40 cfu per ml and from controls 54 cfu per ml (t-test = 0.520, df = 23, p = 0.608) (Appendix Figure 5).

There was no significant difference in the detection of Enterococci from private water supplies belonging to cases and controls 37% and 27% respectively (chi square = 0.999, df = 1, p = 0.318) (Table 55). Likewise there was no significant difference in the number of Enterococci detected in positive private water supplies from cases and controls. The mean number of Enterococci detected in positive samples from cases was 32 cfu per ml and from controls 20 cfu per ml (t-test = 0.687, df= 23, p = 0.499).

Table 53: Detection of coliforms in private water supplies sampled from cases and controls.

Coliform detected	**Case (%)**	**Control (%)**
Yes	27 (63)	21 (62)
No	16 (37)	13 (38)
Total	**43**	**34**

Table 54: Detection of *E. coli* in private water supplies sampled from cases and controls

***E. coli* detected**	**Cases (%)**	**Controls (%)**
Yes	18 (42)	7 (21)
No	25 (5)	27 (79)
Total	**43**	**34**

Table 55: Detection of Enterococci in private water supplies sampled from cases and controls

***Enterococci* detected**	**Cases (%)**	**Controls (%)**
Yes	16 (37)	9 (27)
No	27 (63)	25 (73)
Total	**43**	**34**

5.4 Relationship between the detection of coliforms in a private water supply and the detection of *E. coli,* Enteroccoci and *Campylobacter.*

As would have been expected there was a significant relationship between the detection of coliforms and *E. coli* from private water supplies (chi-square = 22.36, df = 1, p < 0.001). *E. coli* were detected from 52% of the supplies which were positive for coliforms (Appendix Table 57)

There was a significant relationship between the detection of coliforms and Enterococci from private water supplies (chi square = 13.873, df = 1, p < 0.001) (Appendix Table 58). There were only two supplies from which Enterococci were detected in which no coliforms were detected.

All three samples that were positive for *Campylobacter* were also positive for coliforms. Two of the three were also positive for both Enterococci and *E. coli* (Table 56).

Table 56: Relationship between detection of *Campylobacter,* and coliforms, *E. coli* and Enterococci

Sample	Coliform	*E. coli*	Enterococci
Supply 1	+ve 14 cfu per ml	-ve	-ve
Supply 2	+ve 59 cfu per ml	+ve 12 cfu per ml	+ve 61 cfu per ml
Supply 3	+ve 200+ cfu per ml	+ve 66 cfu per ml	+ve 12 cfu per ml

5.5 Seasonality trends in microbial detection from private water supplies

The relatively small number of private water supplies tested each month makes the identification of trends throughout the year difficult. For coliforms there may possibly be a lower percentage of positive samples at the start of the year (Appendix Figure 6). Similarly for *E. coli* the identification of seasonality is difficult, but none of the ten samples tested in January or February were positive for *E. coli (*Appendix Figure 7). For Enterococci there also appears to be a lower detection rate at the start of the year (Appendix Figure 8). *Campylobacter* was isolated from private water supplies on three occasions, once in April, once in December and once in October. These small numbers make the identification of any seasonal trends impossible.

5.6 Relationship between category of private water supply and water testing results

For one private water supply sampled information was not available on the category of the supply.

There was no significant relationship between the detection of coliforms and the category of private water supply. Coliforms were detected in 65% of samples from category B supplies and 40% of samples from category A supplies (Fishers Exact test (2 tailed) p = 0.351) (Table 57).

There was no significant relationship between the detection of *E. coli* and the category of private water supply, detected in 34% of samples from category B supplies and 20% of samples from category A supplies (Fishers Exact test (2-tailed) p = 1.00), (Table 58)

There was no significant relationship between the detection of Enterococci and the category of private water supply, detected in 34% of samples from category B supplies and 20% of samples from category A supplies, (Fishers Exact test (2-tailed) p = 1.000) (Table 59)

Two of the samples which were positive for *Campylobacter* were from category B supplies. No information was available on the category of supply for the third *Campylobacter* positive supply.

Table 57: Coliforms detected and the category of the private water supply

Coliforms	**Category A (%)**	**Category B (%)**
Yes	2 (40)	46 (65)
No	3 (60)	25 (35)
Total	**5**	**71**

Table 58: *E. coli* detected and the category of the private water supply

E. coli	**Category A (%)**	**Category B (%)**
Yes	1 (20)	24 (34)
No	4 (80)	47 (66)
Total	**5**	**71**

Table 59: Enterococci detected and the category of the private water supply

Enterococci	**Category A (%)**	**Category B (%)**
Yes	1 (20)	24 (34)
No	4 (80)	47 (66)
Total	**5**	**71**

5.7 Relationship between treatment of the private water supply and water testing results

From the questionnaire for participants that supplied a Yes or No response to whether the private water supply was treated. There was a significant difference in the detection of coliforms between treated and untreated supplies, detected in 80% of untreated supplies compared to 32% of treated supplies (chi-square = 14.12, df = 1, p < 0.001) (Table 60).

There was no significant difference between the mean number of coliforms detected in samples from supplies reported to be treated and untreated, with a mean of 75 and 97 cfu per ml respectively (t-test 0.617, df=37 p = 0.541).

There was a significant difference in the detection of *E. coli* between treated and untreated supplies, detected in 52% of untreated supplies compared to 9% of treated supplies (chi-square = 10.37, df = 1, p = 0.001) (Table 61)

There was no significant difference between the mean number of *E. coli* detected in private water supplies reported to be treated and those not treated, with a mean of 1 and 47 cfu per ml respectively (t-test 0.978, df = 21, p = 0.339).

There was no significant difference in the detection of Enterococci between treated and untreated supplies, detected in 40% of untreated supplies and 18% of treated supplies (chi-square = 3.09, df = 1, p = 0.079) (Table 62).

There was no significant difference between the number of Enterococci detected in private water supplies reported as treated and those not treated, with a mean of 2 and 37 cfu per ml respectively (t-test 1.276, df = 18, p = 0.157).

For the three supplies that tested positive for *Campylobacter,* two were not treated and information was not provided for the third.

Table 60: Detection of coliforms in treated and untreated private water supplies

Coliforms	**Supply treated**			
	Missing answer (%)	**No (%)**	**Yes (%)**	**Not sure (%)**
No	1 (20)	8 (20)	15 (68)	4 (44)
Yes	4 (80)	32 (80)	7 (32)	5 (56)
Total	**5**	**40**	**22**	**9**

Table 61: Detection of *E. coli* in treated and untreated private water supplies

E. coli	**Supply treated**			
	Missing answer (%)	**No (%)**	**Yes (%)**	**Not sure (%)**
No	3 (60)	19 (48)	20 (91)	8 (89)
Yes	2 (40)	21 (52)	2 (9)	1 (11)
Total	**5**	**40**	**22**	**9**

Table 62: Detection of Enterococci in treated and untreated private water supplies

Enterococci	**Supply treated**			
	Missing answer (%)	**No (%)**	**Yes (%)**	**Not sure (%)**
No	1 (40)	24 (60)	18 (82)	7 (78)
Yes	3 (60)	16 (40)	4 (18)	2 (12)
Total	**4**	**40**	**22**	**9**

CHAPTER 6: MLST RESULTS

6.1 Species, clonal complexes and sequence types isolated

Information regarding MLST type was available for 88.7% (700/789) of the cases in the study. This data included information on species, sequence type and clonal complexes.

- Sequence Type (ST) for *Campylobacter* isolates: Its allelic profile for a standard set of seven housekeeping genes.

- Clonal Complex (CC): A group of STs whose members are linked to at least one other member by being identical for six of the seven MLST genes

C. jejuni was the most frequently identified species, accounting for 94.1% (659) of isolates. The remaining 5.9% (41) were *C. coli.* No isolates of other much rarer species of *Campylobacter* were isolated (Table 63).

Among the 700 isolates, 167 different STs were identified, 16 of which were isolated on ten or more occasions and the remaining 151 - on less than ten occasions. 107 STs were identified on just one occasion. ST 21 was the most prevalent type isolated from 12.1% (85/700) cases (Figure 15).

Among the 700 isolates, 62 different CCs were identified, 11 of which were isolated on ten or more occasions, the remaining 51 - on less than ten occasions. 31 CCs were identified on just one occasion. ST21 was the most prevalent CC, accounting for 25.3% (177/700) of all isolates, and was more than twice as prevalent as the second most prevalent complex ST-45 complex which was isolated from 11.0% (77/700) cases (Figure 16).

Table 63: Species of *Campylobacter* isolated

Species	**Number of cases (%)**
C. jejuni	659 (94.1)
C. coli	41 (5.9)
Total	700

Figure 15: Common Sequence Types of *Campylobacter* isolated from cases (n=700)

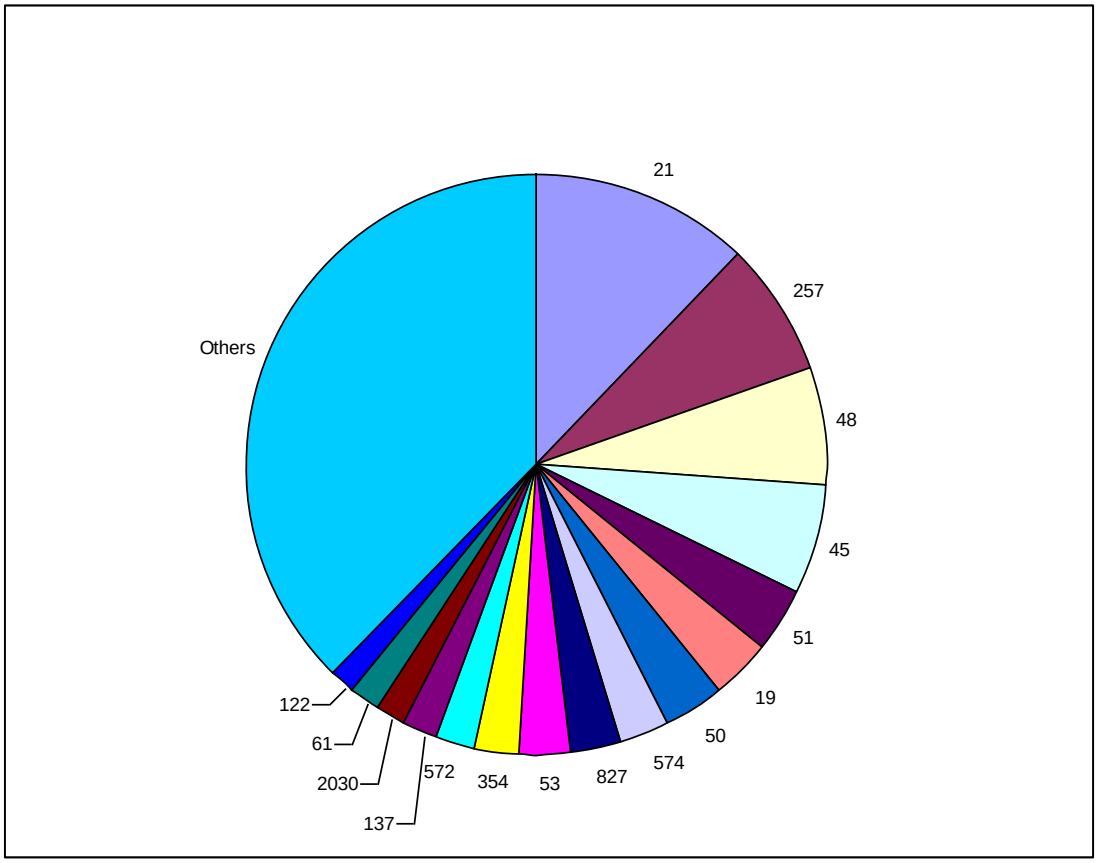
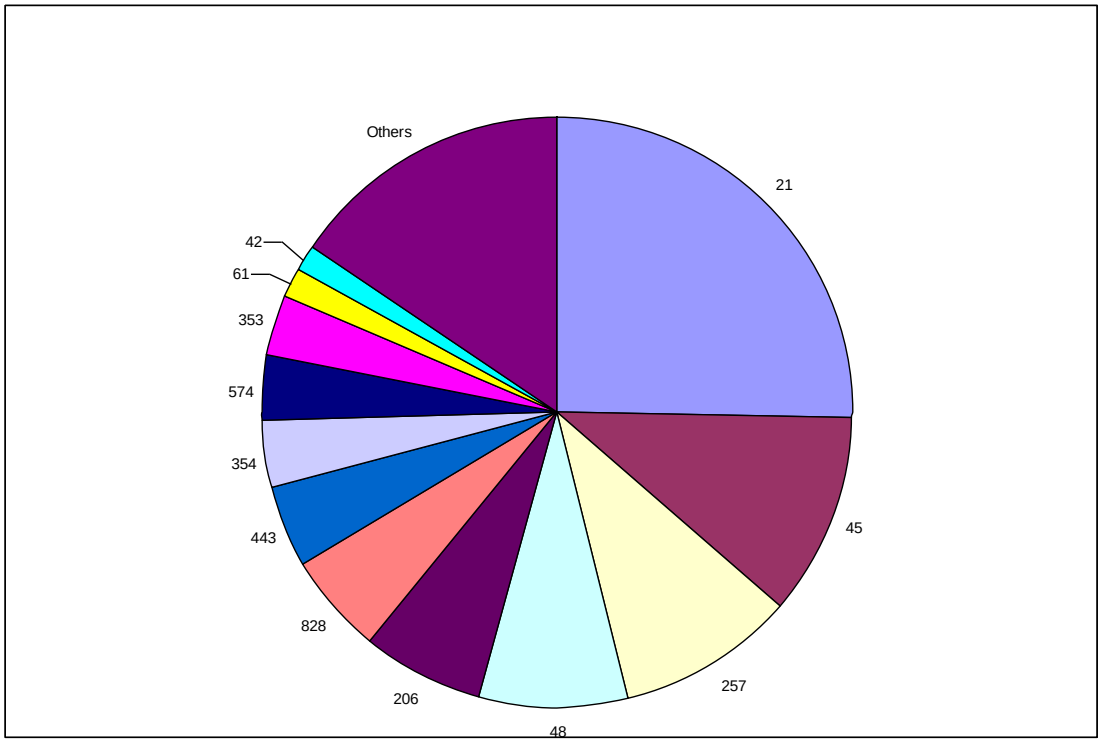


Figure 16: Common Clonal Complexes of *Campylobacter* isolated from cases (n= 700)



6.2 Cases infected with *Campylobacter* on more than one occasion

There were four cases who each participated in the study on two occasions, as a result of being infected on two occasions.

For two of these cases, MLST typing was only available for one of their two isolates. For the other two cases MLST typing was available for both of their isolates. For both cases their second infection was with a strain of different ST and CC than their first infection.

6.3 Cases of *Campylobacter* infection resident at the same address

There were nine households in which more than one laboratory confirmed case of *Campylobacter* infection participated in the study. For two of these households information on the MLST of the isolates was only available for one of the two cases in the household, so no comparison of cases in these households was possible

For the five of the seven households with more than one case participating in the study, the isolates from all cases in the household were of the same MLST. In all five of these households the isolates belonged to CC 21, in three households these were ST 21 and in the other two were ST 50. In two of the seven households the isolates were of different MLST types (Table 64)

Table 64: MLST types for cases resident at the same address (where typing was available for all cases participating from that address).

Household	Case	ST	CC	Same or different
1	1 2	50 50	21 21	Same
2	1 2	21 21	21 21	Same
3	1 2	21 21	21 21	Same
4	1 2	21 21	21 21	Same
5	1 2	574 48	574 48	Different
6	1 2	262 206	21 206	Different
7	1 2 2	50 50 50	21 21 21	Same

6.4 MLST results for water isolates and clinical cases

Campylobacter were isolated from three water supplies: two from cases and one from a control.

For both cases, the clinical isolates were ST 45 and CC 45 and in both incidences this was different from the *Campylobacter* isolated from the water supply. In one incidence the private water supply isolate was ST 1286, in the other the private water supply isolate was ST1614 and CC828 (Table 65).

Using the host attribution data from the MLST study, neither of the strains isolated from water had an attribution to poultry, but rather both to some degree had some attribution to pigs (Table 66).

Table 65: Comparison between clinical isolate from case and isolate from the case's water supply

	Source	ST	CC
Water ID 68	Water	1286	
	Case	45	45
Water ID 328	Water	1614	828
	Case	45	45

Tables 66: Likely source of *Campylobacter* isolated from water

ST	Likely attribution by ST[1]	Likely attribution by allele[2]
1286	Not found	Pig (0.838), wild bird (0.117)
1614	3 strains from poultry, 2 from sheep	Pig (0.558), sheep (0.446)

[1] estimated from animal sources in the CaMPS environmental database
[2] estimated from the CaMPS environmental database using STRUCTUR (allele frequency)
Data provided by University of Aberdeen.

6.5 Relationship between CC and categorical variables.

A number of techniques were employed to investigate the relationship between MLST profile and a number of the variables used in the study.

6.5.1 Analysis using Pearson Chi-square and Likelihood ratio chi-square

For this analysis, due to the large number of CC, many with small numbers of isolates, the analysis was conducted using CC ST 206, ST 21, ST 257, ST 45, ST 48, ST 828 and the remaining CC grouped into other (Appendix Table 59).

6.5.2 Relationship between CC and month of onset of illness

There was a significant association between month of illness and CC. It would appear that, for example, there were more cases of ST45 in May/June than expected and less in Nov/Dec. (Pearson Chi-square = 47.9, DF = 30, p = 0.020), (Likelihood Ratio chi-square = 48.7, DF = 30, p = 0.017) (Appendix Table 60).

6.5.3 Relationship between CC and overnight stay outside the study area and travel abroad

There was a significant association between having an overnight stay outside the study area and CC. More cases of ST-257 had an overnight stay outside the study area than expected (Pearson chi-square = 20.4, DF = 6, p = 0.002) (Likelihood ratio chi-square = 21.8, DF = 6, p = 0.001) (Appendix Table 61). Likewise there was a significant association between travel abroad and CC type (Pearson chi-square = 39.159, DF = 6, p = < 0.001) (Likelihood ratio chi-square = 40.200, DF = 6, p < 0.001) (Appendix Table 62).

6.5.4 Relationship between CC and contact with farm animals

The association between CC type and contact with farm animals was highly significant (Pearson chi-square = 25.234, DF= 6, p < 0.001) (Likelihood ratio chi-square = 24.388, DF= 6, p < 0.001) (Appendix Table 63).

6.5.5 Relationship between CC and eating out

There was a significant association between CC type and eating out (Pearson chi-square = 16.128, DF = 6, p = 0.013) (Likelihood ratio chi-square = 16.338, DF = 6, p = 0.012) (Appendix Table 64).

Using this methodology no significant relationship was detected between CC and hospitalisation (p = 0.650), pets in the household (p = 0.109), chicken eaten outside the home (p = 0.245), eating chicken prepared at home (p = 0.543), eating red meat prepared at home (p = 0.152) and having a private water supply (p = 0.402)

6.6 Differences between exposed *versus* unexposed cases, in the ST composition and host attribution of their *Campylobacter* strains

Differences were analysed in *Campylobacter* strain composition between cases exposed *versus* unexposed to ten main exposure characteristics and between those reporting *versus* those not reporting three of the clinical symptoms (bloody diarrhoea, vomiting and abdominal pain) and hospitalised *versus* non hospitalised cases. Strain composition was quantified at ST and CC/ST levels.

Two of the exposure factors showed evidence for overall differences in strain composition between exposed *versus* unexposed cases. Cases with an overnight stay abroad differed from cases with no overnight stay outside the study area for both ST and CC/ST strains whereas those with an overnight stay within the UK did not (Table 67) Furthermore, cases with an overnight stay abroad had higher levels of ST diversity (DI = 0.981, 95% CI 0.972-0.989) than either cases with no overnight stay outside the study area (DI = 0.957, 95% CI 0.950-0.965) or case with an overnight stay within the UK (DI = 0.948, 95% CI 0.927-0.969) and a similar difference were evident for CC/ST strain diversity. These results were taken into account and cases with an overnight stay abroad were excluded from subsequent comparisons. Cases with contact with farm animals differed from those without contact but only for CC/ST strains (Table 67).

The two exposure factors: an overnight stay abroad and contact with farm animals, that showed statistically significant evidence of differences in overall strain composition between exposed *versus* unexposed cases were analysed further to identify the strains with the largest frequency differences between exposed *versus* unexposed cases. Two criteria for evaluating statistical significance for strain frequency differences were used: a Fisher's exact test *P*-value of less than 0.05 for each strain, which was the less stringent criterion, and a false discovery rate analysis that took account of the *P*-values from all the tests of non-singleton strains, which was the more stringent criterion.

The less stringent statistical criterion yielded the following results. Regarding an overnight stay abroad – nine ST and two CC were more common in exposed *versus* unexposed cases, three ST and two CC strains were rarer in exposed *versus* unexposed cases. Regarding contact with farm animals – two ST and one CC strain were more common in exposed *versus* unexposed cases, and one ST strain was rarer in exposed *versus* unexposed cases (Table 68) Nine of the ST and the four CC strains identified in the overnight stay abroad exposure analysis, and only the CC strain identified in the contact with farm animals exposure analysis, showed significant frequency differences according to the more stringent statistical criterion (Table 68, P values in bold).

There was no evidence for overall difference in strain composition between cases with or without the three clinical symptoms and those admitted or not admitted to hospital (Table 67). Nonetheless, the strains with the largest frequency differences between these groups were identified according to the two statistical criteria described above. The less stringent statistical criterion yielded the following results (Table 69). Regarding abdominal pain – five ST and two CC strains were rarer in those with abdominal pain compared to those without. Regarding bloody diarrhoea – three ST strains and one CC strain were more common in those with bloody diarrhoea compared to those without, and one ST and one CC were rarer in those with bloody diarrhoea compared to those without. Regarding vomiting – one CC strain was more common in those with vomiting compared to those without. Finally regarding hospitalised *versus* non hospitalised cases – one ST and two CC strains were more common. In general the CC strains consisted of the identified ST strains amalgamated with further unidentified STs. Only three of the ST and one of the CC strains identified in the abdominal pain analysis showed significant frequency differences according to the more stringent statistical criterion (Table 69 P values in bold).

Differences in source attribution were associated with four exposure factors, overnight stay outside the study area, contact with farm animals, eating out and water supply. For the reasons given above, overnight stay outside the study area was analysed using all cases, then subsequent factors were analysed excluding cases that had an overnight stay abroad. In comparison to cases with no overnight stay outside the study area, those with an overnight stay within the UK had a higher attribution to ruminants and those with an overnight stay abroad had a higher attribution to wild birds (Table 67). Cases

with a contact with farm animals and cases with a private water supply both had higher attributions to ruminants and lower attributions to chicken than cases without exposure to such factors. Cases eating out (but not reporting eating chicken) had a lower attribution to wild birds than cases not eating out (Table 67). No other differences in source attribution between single factors were identified.

The source attributions of cases exposed to chicken were re-analysed by joint consideration of chicken eaten outside the home and chicken prepared at home. There was no evidence for any differences in attributions values for the four source types among cases not exposed to chicken by either factor, cases exposed to one factor only and cases exposed to chicken by both factors. The 95% confidence intervals of source attribution values in all four exposure categories showed considerable overlap (Table 70).

In summary, out of the ten exposure factors, three clinical symptoms and hospitalisation, only an overnight stay abroad and contact with farm animals were associated with differences in ST and CC/ST strains. Individual strains were both more and less common in cases with exposure to each factor, and the level of strain association was much stronger for those with an overnight stay abroad. Only contact with farm animals and private water supply were associated with differences in host attribution, with both factors showing higher ruminant and lower chicken attributions. There was limited evidence for abdominal pain being reported less often following infection with two rare *C. jejuni* strains or *C. coli* CC828 strains. There was no evidence that exposure to chicken either eaten out or prepared at home or both was associated with any increased source attribution to chicken.

Table 67: Comparison of strain composition and source attributions between cases exposed *versus* unexposed to different infection risk exposures, and cases with *versus* without different disease symptoms/outcomes.

					Differences between "Yes" and "No" groups					
Factor	**No. cases**		**No. of strains**		**Strain composition**		**Source attribution**			
	Yes	**No**	**ST**	**CC/ST**	**ST**	**CC/ST**	**Ruminant**	**Chicken**	**Pig**	**Wild bird**
(a) **Travel cases**										
Travel within GB (Y) vs. no travel (N)	122	456	129	50	Ns	Ns	**H, 0.013**	Ns	ns	ns
Travel overseas (Y) vs. no travel (N)	118	456	152	59	**D, $<10^{-5}$**	**D, 0.001**	Ns	Ns	ns	**H, <0.001**
(b) **Overseas travel cases excluded**										
Pet owner	290	290	129	50	Ns	Ns	Ns	Ns	ns	ns
Farm animal contact	59	419	114	44	Ns	**D, 0.002**	**H, <0.001**	**L, <0.001**	ns	ns
Ate out - chicken (Y) vs. ate in (N)	126	240	99	43	Ns	Ns	Ns	Ns	ns	ns
Ate out - not chicken (Y) vs. ate in (N)	140	240	95	43	Ns	Ns	Ns	Ns	ns	**L, 0.018**
Chicken prepared at home	388	135	122	47	Ns	Ns	Ns	Ns	ns	ns
Red meat prepared at home	294	192	119	46	Ns	Ns	Ns	Ns	ns	ns
Cooked meat prepared at home	302	197	116	46	Ns	Ns	Ns	Ns	ns	ns
Salad prepared at home	323	183	113	45	Ns	Ns	Ns	Ns	ns	ns
Shellfish prepared at home	34	451	114	45	Ns	Ns	Ns	Ns	ns	ns
Household on private water supply	53	528	129	50	Ns	Ns	**H, 0.004**	**L, 0.003**	ns	ns
Case had abdominal pain	535	33	128	49	Ns	Ns	Ns	Ns	ns	ns
Case had bloody stools	185	305	108	44	Ns	Ns	Ns	Ns	ns	ns
Case had vomiting	216	358	128	50	Ns	Ns	Ns	Ns	ns	ns
Case was hospitalised	69	510	129	50	Ns	Ns	Ns	Ns	ns	ns

Notes: D, "Yes" and "No" cases differed significantly. The value following D indicates the *P*-value for the null hypothesis of no difference. H, "Yes" cases had a significantly higher attribution; L, "Yes" cases had a significantly lower attribution; ns, nonsignificant difference. The value following H or L indicates the proportion of 1000 randomised differences more extreme than the observed difference, and <0.001 indicates the observed value was the most extreme.

Table 68: *Campylobacter* strains yielding evidence for frequency differences between cases exposed *versus* unexposed to overseas travel and contact with farm animals.

Factor	Strain	No. of cases No	Yes	Odds ratio [a]	95% CI of odds ratio	*P*-value [b]
Overnight stay abroad						
	ST50	13	10	4.05	1.73 - 9.48	**0.0021**
	ST227	0	3	infinity		**0.0047**
	ST460	0	3	infinity		**0.0047**
	ST464	2	4	10.18	1.84 - 56.22	**0.0088**
	ST572	7	8	5.97	2.12 - 16.81	**0.0012**
	ST824	0	2	infinity		0.0282
	ST883	0	2	infinity		0.0282
	ST2065	0	3	infinity		**0.0047**
	ST2331	0	3	infinity		**0.0047**
	CC206	28	17	3.33	1.76 - 6.31	**0.0006**
	CC460	1	4	20.39	2.26 - 184.08	**0.0034**
	ST21	79	6	0.34	0.14 - 0.80	**0.0082**
	ST45	40	2	0.23	0.06 - 1.00	0.0316
	ST257	53	1	0.08	0.01 - 0.62	**0.0009**
	CC45	72	5	0.31	0.12 - 0.79	**0.0091**
	CC257	66	3	0.20	0.06 - 0.66	**0.0020**
	Total	582	118			
Contact with farm animals						
	ST19	12	5	3.14	1.07 - 9.26	0.0463
	ST827	11	5	3.43	1.15 - 10.26	0.0361
	CC828	20	8	3.13	1.31 - 7.47	**0.0141**
	ST257	41	1	0.16	0.02 - 1.18	0.0459
	Total	419	59			

Notes: Cases with an overnight stay abroad were excluded from the farm animal contact analysis.

a: The odds ratio expresses the relative frequency of an ST in the "Yes" cases in comparison to its frequency in the "No" cases, and is expressed as "zero" or "infinity" when one of the "No. of cases" cells is empty.

b: *P*-values are from Fisher's exact tests of 2x2 contingency tables of each strain, and the ST and CC strains were analysed separately. All the *P*-values less than 0.05 are shown, and those in bold were judged significant after FDR correction for the tests performed on the non-singleton STs or CC/STs.

Table 69: *Campylobacter* strains yielding evidence for frequency differences between symptomatic *versus* asymptomatic cases, and hospitalised *versus* non-hospitalised cases.

Factor	Strain	No. of cases No	Yes	Odds ratio [a]	95% CI of odds ratio	*P*-value [b]
Case had abdominal pain						
	ST38	2	2	0.06	0.01 - 0.43	**0.0183**
	ST267	2	4	0.12	0.02 - 0.66	0.0424
	ST273	2	2	0.06	0.01 - 0.43	**0.0183**
	ST825	2	4	0.12	0.02 - 0.66	0.0424
	ST827	5	14	0.15	0.05 - 0.45	**0.0031**
	CC283	2	4	0.12	0.02 - 0.66	0.0424
	CC828	7	27	0.20	0.08 - 0.50	**0.0020**
	Total	33	535			
Case had bloody stools						
	ST53	5	9	3.07	1.01 - 9.30	0.0496
	ST61	3	8	4.55	1.19 - 17.37	0.0241
	ST574	7	11	2.69	1.02 - 7.07	0.0473
	CC61	3	8	4.55	1.19 - 17.37	0.0241
	ST19	16	2	0.20	0.04 - 0.87	0.0231
	CC828	25	5	0.31	0.12 - 0.83	0.0182
	Total	305	185			
Case had vomiting						
	CC257	33	32	1.71	1.02 - 2.88	0.0425
	Total	358	216			
Case was hospitalised						
	ST607	0	2	infinity		0.0140
	CC61	8	4	3.86	1.13 - 13.18	0.0436
	CC607	1	2	15.19	1.36 - 169.84	0.0388
	Total	510	69			

Notes: Cases travelling overseas were excluded from these analyses.

a: The odds ratio expresses the relative frequency of an ST in the "Yes" cases in comparison to its frequency in the "No" cases, and is expressed as "zero" or "infinity" when one of the "No. of cases" cells is empty.

b: *P*-values are from Fisher's exact tests of 2x2 contingency tables of each strain, and the ST and CC strains were analysed separately. All the *P*-values less than 0.05 are shown, and those in bold were judged significant after FDR correction for the tests performed on the non-singleton STs or CC/STs.

Table 70: Source attributions of cases exposed *versus* unexposed to chicken consumption from both eating out and chicken prepared at home.

	Chicken exposure		Source attribution values				
No. of cases	**Ate chicken out**	**Prepared chicken at home**		**Ruminant**	**Chicken**	**Pig**	**Wild bird**
86	No	No	**Mean**	**0.487**	**0.445**	**0.001**	**0.068**
			U-CI	0.553	0.540	0.013	0.135
			L-CI	0.336	0.361	0.000	0.048
256	No	Yes	**Mean**	**0.463**	**0.467**	**0.003**	**0.094**
			U-CI	0.501	0.493	0.006	0.111
			L-CI	0.413	0.407	0.000	0.068
32	Yes	No	**Mean**	**0.432**	**0.449**	**0.001**	**0.127**
			U-CI	0.620	0.604	0.028	0.173
			L-CI	0.296	0.299	0.000	0.020
83	Yes	Yes	**Mean**	**0.469**	**0.444**	**0.001**	**0.86**
			U-CI	0.546	0.542	0.013	0.135
			L-CI	0.368	0.363	0.000	0.047

Notes: Mean, mean observed source attribution values for a given group of cases; U-CI and L-CI, respectively, upper and lower 95% confidence intervals of host attribution values for a group of cases of the same size as the observed group that was randomly sampled without replacement from a total of 457 cases giving “Yes” or “No” responses to both questions about the two foodborne exposures to chicken, with cases travelling overseas excluded.

CHAPTER 7: DISCUSSION

This study investigated whether the consumption of water from private supplies is a risk factor for *Campylobacter* infection in Aberdeenshire and Aberdeen City. This has involved the collection of epidemiological data, the testing of water quality in both mains and private water supplies from cases and controls and the consideration of MLST data.

7.1 Study design and participation

The study was a case control study. All laboratory confirmed case of *Campylobacter* infection resident in Aberdeenshire and Aberdeen City were invited to participate in a postally administered questionnaire. Controls were frequency matched and selected at random from the Community Health Index (CHI). The overall participation rate for cases was 59.8%, comparable to that reported from other *Campylobacter* case control studies - 61.6%, 62%, 63% and 74.2% (Evans *et al* 2003, Effler *et al* 2001, Friedman *et al* 2004, Stafford *et al* 2007 respectively). The study of Stafford *et al*, which recruited 74.2% of eligible cases, used a telephone administered questionnaire, and hence had a higher participation rate than with postal based questionnaires.

As with any study recruiting microbiologically confirmed cases, these were just a sub-set of all cases. For a case to be invited to participate in this study, the person must have sought medical attention, the clinician must have requested a stool sample and the patient must have actually submitted one, the laboratory must have isolated *Campylobacter* and reported this to the Health Protection Team. Generally patients are probably more likely to consult their clinicians, and clinicians are probably more likely to take specimens from patients, if they are severely ill, not recovering quickly, particularly vulnerable e.g. the elderly, the young, the pregnant, hospital patients, and the immunocompromised. These groups could therefore be over-represented in the study. The IID study in England estimated that for every case reported through national surveillance another 7.6 go unreported in the community (Wheeler *et al* 1999). We do not know if the cases who did not participate in our study had any characteristics or demographics that differed from participating cases. As with similar studies the inability to recruit all cases is a limitation however the participation rate was similar to other studies.

For the 97% of cases where it was possible to calculate the length of time between onset and completing the questionnaire, the mean was 16 days, with 68% of case questionnaires being completed within 14 days or less of onset. It was suggested that data quality might decline with increased time since onset, thus completeness of the questionnaire was compared for cases completing it within and beyond 14 days of onset (Appendix Table 12). For most variables the rate of missing answers was similar in both groups, although more missing answers were noted for questions regarding ‘eating outside the home’ and ‘eating chicken prepared at home’. Likewise for those questions answered as ‘not known/not sure’ where this option was available, the values were generally similar, although again, there were differences for some variables. Despite these differences there was no great loss in completeness of data for those participants completing questionnaires more

than 14 days after onset, therefore it was decided to include all cases in the analysis. In general, the percentages of missing answers or those reported as not known were greater among cases than controls (Appendix Table 13). This was not surprising, as cases were asked to recall the period prior to onset: an average of 16 days, while controls were only asked to recall for the majority of questions the previous five days. As with any such study it was not possible to validate the accuracy of any answers provided by participants or eliminate the possibility of selective recall for some factors.

From controls invited to participate in the study and from whom the information pack was not returned as undeliverable, the overall participation rate was 37%. The participation rate among controls varied considerably with age and gender (Figure 3). The lowest participation rates were in young males in particular those aged 20-29 years. To compensate for this low participation rate and to help to achieve the matching frequency of 2:1, additional subjects within these age groups were selected at random from the CHI, sent a study information pack, and invited to participate. Despite this, the matching frequency in a few groups remained below 2:1, however the overall matching frequency was 2.57:1 for females and 2.25:1 for males and allowed all the analysis originally planned to be conducted and the study to retain its statistical power.

7.2 Demographics; age, deprivation and rural/urban residence

The rate per 100,000 for cases participating in the study was similar to that observed for all laboratory confirmed cases of *Campylobacter* infection in Grampian (Figures 5 & 6) albeit at a lower rate, as not all cases wished to participate in the study. It is notable that among males aged 20-24 years, the rate for confirmed cases in Grampian was 186.9 per 100,000, compared to 89.2 per 100,000 for those participating in the study. This was the largest difference between overall rates and study rates. This further reflects the difficulty in achieving participation among young males, not only as controls as previously discussed but also as cases.

The incidence of *Campylobacter* infection reported in Grampian shows a distinctive age distribution, with peaks among young children (0-4 years) especially among males and a second peak in young adults (20-30 years) (Figure 5). This age distribution of cases is similar to that observed for the whole of Scotland and elsewhere. In the North West of England the highest incidence was in males 0-4 years (Sopwith *et al* 2003). Similarly in Québec, Canada the highest rate was among children 0-4 years with a second peak in young adults 15-34 years (Michaud *et al* 2004). This bimodal pattern has also been reported from America and Germany (Samuel *et al* 2004, Gűrtler *et al* 2005). In contrast with other studies the peak in young children was not observed in the Netherlands where the highest percentage to test positive for *Campylobacter* was amongst those aged 5-9 years (de Wit *et al* 2001).

Among young children (0-4 years) the rate of *Campylobacter* infection among males was considerably higher than among females 187.0 and 75.6 per 100,000 respectively for all laboratory confirmed cases in Grampian (Figures 5 & 6). Additionally participating in the study in the under one years group,

there was a total of six cases all of whom were male (Appendix Table 5). Although the rates for the 10-14 years group were the lowest among all age groups, they also show a large difference between the genders 54.7 and 25.6 per 100,000 for males and females respectively. This difference in rates between males and females has been reported elsewhere (Strachan *et al* 2008) but the reasons for these differences remain to be established. A number of factors could potentially contribute to these differences including food preparation and consumption habits, contact with the environment, and seeking medical attention. For very young children (under one year) food consumption and seeking medical attention is governed by parents, we are unable to speculate if parents treat/feed male and female infants differently such as to account for these differences. Much remains to be understood about these differences, other papers have suggested they may be due to physiological factors (Strachan *et al* 2008) or immunological competence in males (Green 1992).

When considering the distribution of cases by deprivation category, and comparing to the whole population of Aberdeen City those in deprivation category 6 may have been underrepresented: 14% of the City population were resident in a category 6 area, compared to just 8% of cases (Appendix Tables 6 & 7). The distribution of deprivation categories in the population of Aberdeen City and Aberdeenshire differs from that of the whole of Scotland. There are no category 7 postcodes within the study area, while overall in Scotland 7% of the population are resident in category 7 areas. At the most affluent end of the scale, 16% and 24% of the population of Aberdeen City and Aberdeenshire respectively are resident in category 1 areas, compared to only 6% of the whole population of Scotland (Appendix Table 7). This difference in the population should be taken into consideration when extrapolating the results presented here to the whole of Scotland, especially since socioeconomic status has been reported as a factor associated with gastrointestinal infection in other research. A study of socioeconomic factors and bacterial gastrointestinal infections in Denmark found that high income groups and education were associated with an increased risk of *Campylobacter* infection. The authors suggested that the risk of infection was not primarily associated with poverty but rather with increasing socioeconomic status. They also suggested that differences may be explained by differences in diet and travel activity although may also in part reflect differences in probabilities of diagnostic reporting (Simonsen *et al* 2008). The effect of affluence was also evident in this study. When considering the risk associated by Scottish Index of Multiple Deprivation score an increased risk was associated with higher quintiles in comparison to the lowest quintile (Table 44). From our study it is impossible to determine whether this bias is resulting from those in more affluent areas being more likely to seek medical attention, submit a stool sample or participate in the study. A difference in willingness to participate in the study was unlikely to have been a factor, as controls were not matched by postcode, and any influence of affluence in participating in the study could be expected to be fairly consistent between cases and controls. The affect of affluence, may possibly be linked to other exposures including travel outside the study area, eating chicken outside the home, or other factors.

Cases were significantly more likely to be resident in Aberdeenshire than controls. The study did not employ any selection criteria based on residence, with controls being selected at random from the CHI, therefore this is likely to be a real difference rather than selection bias. This association between infection and living in a rural as opposed to an urban area has been reported in a number of other studies. An earlier study conducted in Grampian (2000 to 2006) using information on cases found a higher incidence in young children (< 5 years) living in rural, compared to urban areas, there was no clear trend for older age groups (Strachan *et al* 2009). A registry based case control study in Denmark, reported that living in types of housing found in rural areas and living in areas with a low population density were both associated with an increased risk of infection (Ethelberg *et al* 2005). In the Danish study the increase in risk in rural areas was primarily carried by children. A study in the Manitoba province in Canada revealed a higher incidence in populations living in rural compared to urban areas (Green *et al* 2006). The Canadian study showed population groups living in rural areas, employed in agricultural occupations and living in geographic areas with exposure to high levels of animal densities had rates of *Campylobacter* infection two to almost three times higher than in lower risk areas.

7.3 Clinical presentation

As would be expected diarrhoea and abdominal pain were reported by over 90% of cases. Vomiting and bloody stools were reported by 37% and 31% respectively (Table 5). 10.7% of cases were admitted to hospital. This was slightly higher than reported in the *Campylobacter* Sentinel Surveillance Study conduced in Lothian (2001-2003) in which 6.6% were admitted to hospital. The Sentinel Surveillance Study in England and Wales reported a hospitalisation rate of 10% (Gillespie *et al* 2003). A study in America reported that 12% of cases were hospitalised (Friedman *et al* 2004), a French study reported 14% were hospitalised (Galley *et al* 2008), while surveillance data from New Zealand report 6% cases were hospitalised (Baker *et al* 2007). Variation in hospitalisation rates may be a reflection of differences in sampling criteria, especially for those at the milder end of the clinical spectrum and possibly participation in the study, as those admitted to hospital may have been more likely to participate in the study.

In this study the rate of hospitalisation was highest among those at the extremes of age (Figure 10). However, results should be considered with caution as the numbers in some age bands were small. Hospitalisation could also be associated with the presence of other underlying conditions especially in the elderly. The study did not collect information on this, but it is something which could possibly warrant further investigation. Interestingly, the prior use of antibiotics was greater in hospitalised compared to non-hospitalised cases. This should be viewed with caution, as although the question asked about antibiotics taken prior to onset, cases may have misinterpreted as antibiotics taken for this infection. Hospitalised cases had a significantly higher rate of bloody stools and vomiting than non-hospitalised cases (Tables 7 & 45), as had previously been reported in England and Wales (Gillespie *et al* 2006). The greater rate of bloody diarrhoea and vomiting, coupled with an

understanding of the age groups with high rates of hospitalisation could act as an important predicator for hospitalisation in the clinical management of such cases.

The fact that 10% of cases in this study were hospitalised highlights the potential seriousness of infection and the considerable resultant social and economic burden. This is also reflected in the average duration of illness of 8.7 days for non-hospitalised cases and 12.2 days for hospitalised cases. This study did not seek to gain any information on complications or sequelae arising from infection, however it is widely recognised that Guillain-Barré syndrome is a rare but serious complication (Tam *et al* 2003).

7.4 General outbreaks

We excluded from the analysis cases that were recognised as part of general outbreaks. The definition of outbreak applied was that used for ObSurv: The surveillance system for all general outbreaks of infectious intestinal disease in Scotland. For the purpose of ObSurv an outbreak is defined as an incident in which two or more linked cases experience the same illness or when the observed number of cases unaccountably exceeds the expected number. A general outbreak is one affecting members of more than one household or residents of an institution. During the study cases were associated with two general outbreaks. In the first of these seven cases were excluded from the study. These cases were part of a larger outbreak occurring in Tayside NHS Board in which a total of 86 persons were ill, 34 of whom were microbiologically confirmed, chicken liver pate was the suspected vehicle of infection in the outbreak (Cowden and Smith-Palmer 2006). The second smaller outbreak excluded three cases from the study, this was an outbreak associated with a restaurant in Grampian in which a total of 12 persons were ill, five of whom were microbiologically confirmed.

7.5 Others in household with similar illness

Most cases of *Campylobacter* infection are generally regarded as sporadic, with general outbreaks being rarely reported (Cowden and Smith-Palmer 2006, Pebody *et al* 1997). ObSurv only captures information on general outbreaks and not single household outbreaks. In this study, 13.7% of those who reported others living in the same household also reported that at least one other person in the household was ill with similar symptoms. This was significantly greater among children than adults. A similar value was reported from the Sentinel Surveillance Study in England and Wales, in which 17% of *C. jejuni* cases who did not live alone reported another individual within the household with similar symptoms (Gillespie *et al* 2003). It is important to recognise that a case reporting household members with similar symptoms does not mean that these individuals were also infected with *Campylobacter.* Their symptoms could have been due to another pathogen or a non-infectious aetiology. Unfortunately our study did not collect information from controls on the members of their household who had gastrointestinal symptoms. This would have been a valuable comparison against the proportion reported by cases. A study in Denmark reported that 3.2% of *Campylobacter* cases were part of household outbreaks, however this study was based on laboratory confirmed cases only and did not take into consideration others in the

household being ill but not laboratory confirmed (Ethelberg *et al* 2004). The comparable value for this study was 2.4% of cases participating in the study belonging to a household in which another case also participated. Among these the difference in dates of onset between the cases in the same household ranged from 0 to 64 days. In four of the nine households the difference between the dates of onset was greater than 14 days (Appendix Table 16), suggesting that these cases may be sporadic cases rather than linked cases.

Interestingly, for cases who do not live alone, having an overnight stay outside the study area (Table 16) contact with farm animals (Appendix Table 24) and having a private water supply were all shown (using chi-square analysis) to be significantly associated with others in the same household being ill with similar symptoms. The finding of an association with farm animals was also reported by Gillespie *et al* (2003), who found an association with illness in the home and visiting a farm in the two weeks before onset, that study also found associations with consuming organic meats in the winter and contact with a pet suffering from diarrhoea.

7.6 Identification of risk factors for *Campylobacter* infection

The analysis of risk and potentially protective factors was conducted primarily using 'well' controls. 'Well' controls were defined as controls who did not report any of the four symptoms (diarrhoea, bloody stools, abdominal pain or vomiting) associated with *Campylobacter* infection. The exclusion of controls with any symptoms likely to be *Campylobacter* infection allowed the exclusion of any unidentified cases from the control group. The unintentional inclusion of cases among the controls could have reduced the strength of association calculated for risk factors. The analysis was also conducted using all controls and very similar results were obtained (Table 41). Analysis was also conducted using only those participants resident in Aberdeenshire, again this was conducted using both 'well' controls and all controls and similar results obtained (Tables 42 & 43).

7.7 Travel outside the study area

This study identified a number of factors that increased the risk of *Campylobacter* infection. One of these factors was travel outside the study area. In the 14 days prior to onset 34.6% of cases reported an overnight stay outside the study area, compared to 20.8% of controls. 18.5% of all cases and 7.7% of controls had a history of overseas travel. Overseas travel has been identified as a risk factor in a number of other studies (Unicomb *et al* 2008, Stafford *et al* 2007, Friedman *et al* 2004, Neimann *et al* 2003, Rodrigues *et al* 2000, Neal & Slack 1997, Eberhart-Phillips *et al* 1997, Schorr *et al* 1994). For those who had travelled outside the study area, 4.0% of cases had visited Africa compared to 1.8% of controls. Travel to Asia was reported by 11.4% of cases, more than three times the percentage of controls (3.0%). The risk of travel to Africa and Asia as well as South America in relation to gastrointestinal illness, rather than specifically *Campylobacter* has been reported in a number of travel medicine specific studies (Smith-Palmer & Cowden 2009, Redman *et al* 2006, Greenwood *et al* 2008).

There are a number of potential reasons as to why overseas travel may be a risk factor for *Campylobacter* infection. Firstly, this may be partly a reporting bias, as cases who have returned from overseas travel may be more likely to seek medical attention and the clinician may be more likely to request a stool sample than for those with no travel history. Secondly, overseas travel may be related to other risk factors such as eating chicken at restaurants, or taking part in other activities. Thirdly, depending on the country/area visited, the standard of hygiene may not be as good as in the UK, with greater potential for contaminated food and water and cross contamination of other foods. Fourthly, cases exposed to *Campylobacter* overseas may be exposed to different strains to those encountered at home. Therefore any immunity they might have acquired to *Campylobacter* may not be protective against strains not previously encountered. This acquisition of different strains was evident from the MLST analysis, with greater strain diversity among cases with a history of travel abroad compared to those without (Tables 67, 68 & Appendix Table 65). It is likely that the risk associated with overseas travel may be a combination of the above and possibly as yet unidentified factors.

7.8 Use of medication

This study did not identify any significant association between the prior use of antibiotics and antacids and *Campylobacter* infection. The lack of association with antibiotics is in contrast to a small study in Hawaii which found that taking antibiotics during the 28 days prior to onset was a significant independent predicator of illness (Effler *et al* 2001). A study in France found antibiotic use to be a risk factor for indigenous ciprofloxacin-resistant *C.jejuni* infection, but not for ciprofloxacin susceptible infection (Galley *et al* 2008). In our study antibiotic susceptibility testing of isolates was not undertaken and therefore it was not possible to determine whether use of antibiotics was a risk factor for an emergence of resistant strains. However, an association was found for adult participants between using omeprazole (Losec) a proton pump inhibitor, cimetidine or ranitidine (Tagamet and Zantac respectively) both H_2-receptor antagonists and *Campylobacter* infection, adjusted odds ratio 2.732 (Table 44). A study by Neal and Slack (1997) found both omeprazole and H_2 antagonists to be risk factors for *Campylobacter* infection. A study of acid-suppressing drugs and the risk of bacterial gastroenteritis found that current use of proton pump inhibitors was associated with increased risk of gastroenteritis, whereas no association was observed with H_2-receptor antagonists (Rodríguez *et al* 2007). In our study, as the question grouped both the proton pump inhibitor and H_2-receptor antagonists together, it was not possible to look at these two groups individually.

7.9 Animal contact

The adjusted odds ratio for contact with farm animals (for well controls & whole study area) of 1.320 (0.939 to 1.855) did not reach the level of statistical significance (Table 44). When the unadjusted odds ratios were calculated for adults and children separately, a significant association was determined for children between contact with farm animals and infection (Appendix Table 23). The importance of contact with farm animals has shown considerable variation in other studies. Direct contact with cows or calves was independently associated with infection in a study in Wales (Evans *et al*

2003). An American study found a risk with contact with farm animals (Friedman *et al* 2004). A New Zealand study reported an association with contact with cattle (Eberhart-Phillips *et al* 1997). A Norwegian study found a risk with contact with farm animals or their faeces, when animal species were analysed separately, daily contact with cattle, sheep or poultry was associated with increased risk of infection (Kapperud *et al* 2003). A study in rural Michigan found that contact with farm animals was a significant risk factor for *C.jejuni* in rural areas and specifically the care and raising of poultry (Potter *et al* 2003). In contrast, in a French study contact with farm animals was not a significant risk factor (Gallay *et al* 2008). Likewise an Australian study found no significant association with contact with any farm animal or native animals nor did it find any significant association with living on a farm or visiting a farm (Stafford *et al* 2007). From the nature of the questions asked in our study it was not possible to look at any risk associated with particular types of farm animals as had been done in the study of Kapperud *et al* (2003). Although the study questionnaire asked those responding 'yes' to the question regarding contact with farm animals, to specify the type(s) of animals, and while many participants did, others reported 'all farm animals' or 'work on farm'.

In this study the risk associated with having a pet in the household was barely significant, adjusted odds ratio 1.238 (1.033 to 1.483) (Table 44). As with contact with farm animals the evidence from previous studies is variable. A French study reported that contact with any pet was not a significant risk factor (Galley *et al* 2008). An American study found no increase in risk associated with contact with various animals (Saeed *et al* 1993). In a Danish study daily contact with pets was only identified as a risk factor in one of the two models applied (Neimann *et al* 2003). While in another study living in a household with a dog was not associated with an increased risk, but living with a cat was marginally so (Kapperud *et al* 2003). In contrast other studies have found an increased risk associated with contact with puppies and / or dogs (Carrique-Mas *et al* 2005, Friedman *et al* 2004, Stafford *et al* 2007, Eberhart-Phillips *et al* 1997, Neal & Slack 1997, Tenkate & Stafford 2001, Salfield and Pugh 1987). The association between human cases and dogs is also supported by a couple of studies in which identical strains where isolated from a human case and pet dog (Damborg *et al* 2004, Wolfs *et al* 2001). This study did not seek to investigate further any potential risks associated with different types of pets, as has been done in some other studies.

7.10 Consumption of chicken inside and outside the home

The two primary variables for chicken consumption considered in this study were 'chicken consumed outside the home' and 'chicken prepared at home'. For chicken consumed outside the home the study questionnaires asked, 'did you eat out or consume food obtained from Carry Out facilities' in the five days before onset/previous five days. Participants were requested to complete a table for where the meal was eaten/obtained, the date and the type of food consumed. From these it was determined whether the participant had eaten chicken prepared outside the home. As this was a free text field it was not always possible to determine if the food included chicken (for 19% of those who had eaten out whether the food included chicken was not specified and for a further 4% information on food eaten was missing [Table 20]) for

example answers could include ‘curry’ or ‘food from buffet’. As the main objective of the study was to investigate the role of private water supplies as a risk factor for *Campylobacter* infection and rather than investigating the risk associated with chicken, the questions regarding chicken consumption were limited in comparison to some other studies and must be taken into consideration when considering these findings. There has been considerable variation from previous studies on the role of chicken as a risk factor and in some studies it was even reported as a protective factor in *Campylobacter* infection.

This study found eating chicken prepared outside the home in a restaurant or from a take away etc., be to risk factor, adjusted odds ratio 2.118 (1.677-2.676) (Table 44). This finding is consistent with that from a number of other studies (Effler *et al* 2001, Rodrigues *et al* 2000, Michaud *et al* 2004, Unicomb *et al* 2008, Friedman *et al* 2004, Eberhart-Phillips *et al* 1997, Evans *et al* 2003, Baker & McLean 2005). There may be a number of possible reasons for this, including the potential for cross contamination, the use of more fresh than frozen chicken which is likely to have higher levels of contamination. It is also possible that there may be some selective recall with those who become ill, associating their illness with food eaten outside the home and viewing chicken as a likely source

This study found no significant association with chicken prepared at home and *Campylobacter* infection, adjusted odds ratio 0.941 (0.768 to 1.152) (Table 44). For 12% cases and 7% controls the answer to eating chicken at home was either missing or ‘not sure’. The finding of no significant association with *Campylobacter* infection and chicken prepared at home is consistent with other studies (Rodrigues *et al* 2000). While others have reported chicken consumption in general and found it to be a risk (Neal & Slack 1997, Evans *et al* 2001). Kapperud *et al* (2003) found eating chicken that had been bought raw was a risk factor. Wingstrand *et al* (2006) identified the main domestic risk factor to be eating fresh, unfrozen chicken. Poultry liver was reported to be risk factor in one study (Schorr *et al* 1994). In contrast Effler *et al* (2002) found eating chicken prepared at home was inversely associated with infection, likewise Eberhart-Phillips *et al* (1997) reported the recent consumption of baked or roast chicken seemed protective.

This study did not explore whether any of the chicken was undercooked, rare etc, which has been reported to be a risk factor in a number of other studies (Michaud *et al* 2004, Stafford *et al* 2007, Eberhart-Phillips *et al* 1997, Neimann *et al* 2003).

The association with consumption of chicken is also supported by biological plausibility, knowing that chicken meat in the UK is regularly contaminated with *Campylobacter* (Meldrum & Wilson 2007, Meldrum *et al* 2006, Jørgensen *et al* 2002).

7.11 Drinking bottled water

The possibility of bottled water being a potential risk factor for *Campylobacter* infection was only identified relatively recently. A study in Wales was the first

to report an association with drinking bottled water (Evans *et al* 2003). A recent study in Australia reported that cases were significantly more likely than controls to have commercial bottled water as their primary source of water (Stafford *et al* 2007) the odds ratio in that study was 1.5 (1.0 to 2.3). The authors believed this association was probably due to confounding with another factor as the association disappeared in the multivariable analysis. A case-case comparison of *C. coli* and *C. jejuni* infection, found persons with *C. coli* infection were more likely to have drunk bottled water than those with *C. jejuni* (Gillespie *et al* 2002). In our study drinking bottled water was a small risk having an adjusted odds ratio of 1.288 (1.068-1.552) (Table 44). The magnitude of the risk was similar to that reported from the Welsh study of 1.98 (1.48 – 2.67) (Evans *et al* 2003). An association with bottled water is biologically plausible as the water could become contaminated with *Campylobacter* or other pathogens. In Europe, legislation requires mineral water to be free from parasites and pathogenic organisms but, unlike tap water, it may not be treated in any way that might alter its chemical composition (Barrell *et al* 2000). Although the questionnaire in our study asked for those drinking bottled water to specify the number of glasses (of still and sparkling water), this was poorly completed with some providing details of bottles drank, therefore we were unable to investigate this factor further in terms of quantities drunk.

7.12 Recreational water activities

The study asked about exposure to six recreational water activities (swimming, canoeing, sailing, fishing, surfing and diving in the sea). Due to the relatively low numbers reporting taking part in any of these activities, they were combined into a single variable for water activities for the determination of adjusted odds ratio (Table 44). Participation in these water activities did not represent a significant risk factor for *Campylobacter* infection. There is limited and inconsistent information available from other studies on water activities in relation to *Campylobacter* infection. Schonberg-Norio *et al* (2004) identified that swimming in natural sources of water was a risk factor. In an Australian study swimming in a hot tub or pond was significantly associated with infection in the ≥ 5 year age group (Unicomb *et al* 2008). Participating in recreational water sports when water was not swallowed, had a borderline statistical significance in a study in England (Rodrigues *et al* 2000). In contrast swimming was independently associated with a decreased risk in a Norwegian study (Kapperud *et al* 2003). The direct comparison between studies is not possible due to differences in the exact variables used when investigating the potential role of recreational water activity. Our study asked about swimming, but did not specify if this was in a swimming pool which is likely to be chlorinated and subject to water quality checks, or in natural water which is open to direct contamination from wild birds and animals and run-off from ground water.

7.13 Private water supplies

The primary aim of the study was to investigate the role of private water supplies as a risk factor for *Campylobacter* infection in Aberdeen City and Aberdeenshire. The study has clearly shown the risk of private water supplies, the adjusted odds ratio (adjusted for age and sex) for all cases in Aberdeen city and Aberdeenshire compared to well controls was 3.062 (95% confidence interval 2.056 – 4.562) (Table 44). The risk associated with private water supplies was maintained even after adjusting for other known risk factors including, travel abroad, farm animal contact, having a pet in the household, drinking bottled water, use of medication, eating chicken both that prepared within the home and that eaten outside the home (Table 44).

This was the first study in the UK to demonstrate and quantify the risk of private water supplies in sporadic *Campylobacter* infection. The risk associated with water has been reported from a few other countries however the variables used, such as drinking undisinfected water, are not directly comparable to the variable of private water supplies used in this study. A study in Norway reported that drinking undisinfected water was a leading risk factor, however this variable also included those who had drunk directly from a surface water source. That study also found cases were more likely than controls to use undisinfected water in their household (Kapperud *et al* 2003). Undisinfected water is not the same as having a private water supply, as some private water supplies are treated (Table 38). The drinking of dug-well water was found to be a risk factor in a study in Finland (Schonberg-Norio *et al* 2004). A study in New Zealand found a strong association with the consumption of untreated rain water. The authors reported that although the association with rainwater as a home water source had not been described elsewhere, it was biologically plausible, as wild birds could easily contaminate these systems by roosting on the roof where the rainwater was collected (Eberhart-Phillips *et al* 1997). To the best of our knowledge none of the private water supplies in our study involved the collection of rain water. A study in Sweden found that having a well in the household was significantly associated with *Campylobacter* infection (Carrique-Mas *et al* 2005). The same study also found a risk with drinking water from a lake/river. Another study in Sweden investigated the geographical distribution of *Campylobacter* infection and found a negative association with the percentage of the population receiving water from a public water supply (Nygård *et al* 2004). A study in Norway which modelled the incidence of domestically acquired *Campylobacter* infection, reported that receiving treated drinking water was protective, however it was recognised in the study that the categorisation of the number of people that received disinfected or non-disinfected water was complicated and was based on modelling rather than case control or cohort methodology (Sandberg *et al* 2006). It is interesting to note that a study in Australia of *Campylobacter* infection in persons aged 5 years and older found no significant association between drinking untreated water and illness (Stafford *et al* 2007), which was in contrast to most other studies in the area.

Our study did not address the consumption of water from a private water supply outside the home. It is important to consider that many more are exposed to private water supplies each year as a result of using such supplies

when visiting friends or family or at campsites, guest houses, etc. The risk of contracting *Campylobacter* infection or indeed any other gastrointestinal infection from private water supplies may be higher in this population as they are not normally exposed to the supply and thus will not have developed any immunity that may potentially result from habitual exposure. Supporting this theory is a Canadian study (Stauss *et al* 2001) of acute gastrointestinal illness and private water supplies that found that both older age and longer duration of residence were both independently associated with a statistically significant lower incidence of gastrointestinal symptoms. The authors proposed that these people had a greater chance of being exposed to contaminated water and hence a greater opportunity to develop resistance/tolerance to a number of enteric pathogens. The potential effort of regular rather than occasional exposure is also highlighted in an outbreak of *E. coli* O157 in which all six cases identified were visitors to the area and the permanent residents were unaffected (Licence *et al* 2001). The determination of risk associated with occasional rather than regular exposure would be difficult to undertake, as those visiting a house on a private water supply may not necessarily be aware that there was a private water supply, other cases may only be exposed while visiting the area and so would not be eligible to participate in the study and would be hard to identify.

Under the Private Water Supplies (Scotland) Regulations 1996, small hotels, B&Bs, campsites and self-catering holiday accommodation are required to ensure their water supply meets the stringent quality standards set by the regulations. Public or commercial premises are also required to display a prominent information notice alerting holiday makers and other consumers to the potential risk associated with water from a private water supply, as was also recommended in the *E.coli* O157 Task Force Report of 2001 (Drinking Water Quality in Scotland 2005). At present no information is available on the extent to which this is adhered to or the impact it is having on the behaviour of holiday markers or others exposed to such supplies. However the provision of such information is not required by those on Type B supplies for friends or family visiting such private houses.

Overall the risk associated with private water supplies remained after adjusting for age and sex. Although, it is interesting to note that in the under 1 years group among cases one had a private water supply and five had a mains supply and for controls none had a private water supply and nine had a mains supply. Eleven of the cases in the 1-4 years group had a private water supply and 36 a mains supply, while only one control had private water supply and 84 a mains supply (Appendix Table 36). Therefore the importance of private water supplies may be even more pronounced in young children who potentially have not yet developed immunity to *Campylobacter* infection. Parents may be unaware of the potential risk to their children of private water supplies and this could be a potential point for targeted advice and education.

This study found no significant seasonal variation in the risk of *Campylobacter* infection associated with private water supplies, when comparing the seasonal pattern of risk for people with private water supplies against the rest. An alternative approach would be to consider any seasonal trend in the level

or prevalence of *Campylobacter* in water samples from private water supplies, however there was insufficient positive supplies to be able to draw any conclusions on seasonality from the water testing component of the study. Contamination of private water supplies may be due to a number of factors, including direct contamination of the water by animal faeces or by run off into the water source. As such contamination may be related to animal grazing and rainfall. Heavy rainfall after a period of dry weather may present a particular risk of run off into private water supplies and as such contamination may be more closely related to weather parameters and farming practices than the season *per se*. None of the other case control studies that have investigated untreated water supplies and other similar variables have reported any seasonal variation of risk associated with the water consumption. Interestingly a study in Norway using ecological modelling found that rainfall was a risk factor for *Campylobacter* infection, but did not demonstrate the route by which this risk was being translated into infection (Sandberg *et al* 2006).

7.14 Potentially protective variables

As well as identifying risk factors for *Campylobacter* infection, the study has also identified factors that appear to be protective, in particular raw vegetables washed in tap water, salads washed in tap water, fruit washed in tap water and salads/raw vegetables eaten at home (it is recognised that the last of these four variables overlap with the first three). From the simple determination of odds ratios for all four variables the level of significance was < 0.001 (Tables 25 & 30 to 32).

A couple of previous studies have reported similar results. A study in England reported that the consumption of fruit, as well as the consumption of pulses and boiled rice was significantly associated with a lower risk of infection (Rodrigues *et al* 2000). While another study found that eating mutton, raw fruits or berries and swimming were independently related to a decreased risk (Kapperud *et al* 2003). An Australian study also reported eating raw salads and vegetables was associated with a reduced risk of infection and this risk was reduced further as the number of different types of raw salad and vegetables food items consumed during the exposure period increased (Stafford *et al* 2007).

It is unclear whether the apparent protection from the consumption of raw vegetables, fruit and salad as reported here, is due to the possible role of vitamins in an immune response or if they are acting as a proxy to other as yet unidentified healthy eating or lifestyle factors or if there is a degree of selection bias in the controls participating in the study being more health conscious and hence having a higher consumption of fruit and vegetables.

No attempt was made in this study to quantify the consumption of fruit, salads, vegetables and poultry. It is important to take into consideration that it is possible that those reporting the consumption of fruit, salad or vegetables are doing so at the expense of consuming poultry and/or other products identified as risk factors. The protective role of fruit, vegetables and salads warrants further investigation, to establish comprehensively if this is a true protective

effect or rather a selection bias or a reduction in chicken consumption or other risk factors. Such research could also consider if any such protective effect is restricted to *Campylobacter* or covers other gastrointestinal pathogens and the potential mechanisms involved.

7.15 Water testing component of the study

The study design was amended after the first year of data collection for the main study, so as to only undertake microbiological water testing for participants on private water supplies. This decision was based on the first year results from the water testing and the almost non-existent contamination of mains samples compared to private water supplies (Tables 49 to 52).

It is likely that even the low level contamination detected from a few mains supplies, was due to contamination of the kitchen tap rather than the water source. Although the taps were cleaned prior to the samples being taken, the design of some taps made this cleaning difficult. All positive mains results were reported to Scottish Water who in return reported no problems with their supplies related to these areas.

The uptake rate for consenting to participate in the water testing component was high, 78% in the pilot and 68% in the main study: even after the information sheets had been amended in year two to only invite those on private water supplies to participate in the water testing component. Although the consent rate among controls was significantly greater than cases 70% and 66%, respectively (Table 46) it was still considered high in both groups. Consenting to participate in the water testing was also significantly higher among parents consenting on the behalf of children than among adults, 76% and 68% respectively (Table 47). There was no significant difference in consenting by residence in Aberdeen City or Aberdeenshire, 70% and 68% respectively. Likewise, there was no significant difference among those on mains or private water supplies, 69% and 68% respectively therefore those on private water supplies were not less likely to participate than those on mains.

Overall water testing was conducted on 1006 samples, 29% from cases and 71% from controls (Table 48). 92% of samples were from mains supplies and 8% from private water supplies, there were four samples taken where the definitive supply was coded as both.

For 287 cases, data were available for the number of days between the onset of illness and the water sample being taken, this ranged from 6 to 153 days, with a mean of 34 days (Figure 14). This delay reflects the time between onset and the case seeking medical attention and submitting a stool sample, time for the laboratory to isolate and report the *Campylobacter,* the public health team to send the study information pack, time for the questionnaire be to completed and returned to HPS, HPS to pass the contact details to Aberdeen University team and for them to contact the case and arrange a mutually suitable time to visit and collect the sample. Therefore the results for the water samples indicate the overall quality of the water source, rather than providing an accurate picture of the water quality in the days prior to onset.

While this is recognised as a weakness of the study, there were no practical ways in which this could be avoided.

Coliforms were detected in only 1.7% of samples from mains supplies compared to 62% of private water supplies (Table 49). The level of contamination among mains supplies was comparable to that in a previously published paper which reported the percentage of samples showing the presence of thermotolerant coliforms in drinking water from public systems in Europe at around 1-2% (van Lieverloo *et al* 2007). In those supplies which were positive, the level of contamination was lower in mains samples compared to private water supplies. Seven of the 16 positive mains supplies had only one coliform compared to none of the 48 positive private water supplies. Enterococci were only detected in one sample from a mains supply (0.1%) compared to 25 (32%) samples from private water supplies. In none of the mains supplies was *E. coli* or *Campylobacter* detected, compared to 25 (32%) and three in private water supplies respectively.

Among private water supplies 62% and 32% were positive for coliforms and *E. coli,* respectively. This was higher than reported in a study which used the results from the statutory testing of private water supplies in nine Public Health Laboratories in England, in which 27% and 21% of samples contained coliforms and *E. coli* respectively (Rutter *et al* 2000). The results were also higher than in a previous study of the quality of drinking water from private water supplies in Aberdeenshire sampled between 1992 and 1998, in which the failure rate was 41% and 30% for total coliforms and faecal coliforms respectively (Reid *et al* 2003). The study of Reid *et al* (2003) also found a similar failure rate for samples collected directly from the source (i.e. well) compared with those taken from the potable tap (usually the kitchen cold water tap), suggesting that it is the ground water source itself that contributes much to the microbiological contamination rather than a problem with the contamination of the storage or supply line. In Scotland, under the Private Water Supplies (Scotland) Regulations 1992, the results of monitoring to the end of 2005 show that 3255 (approximately 35%) of supplies tested failed to meet the requirements of these regulations (Drinking Water Quality in Scotland 2005). It is not known what proportion of supplies sampled at the time would have been now classified as type A or type B, as this could have influenced the percentage of failures. A study of seven large commercial private water supplies in the UK undertaken over two six-week periods in the spring and autumn of 2000, found that all supplies experienced intermittent pathogen presence and only one, a chlorinated deep borehole supply, fully complied with the water quality regulations in force at that time (Kay *et al* 2007). A study of private water supplies in rural communities in Ontario (Canada) found that according to the regulatory standards employed in Canada during the study 17.1% of water supplies exceeded acceptable levels of total coliform and 9.5% exceeded acceptable levels of *E. coli* for at least one sample. (Strauss *et al* 2001).

Our results highlight the extreme difference in the general quality of water from mains supplies and from private water supplies, and also the wide variation in the quality between different private supplies, with no pathogens

being detected in some samples compared to the high numbers in others. However, it must be taken into consideration that in this study, each supply was sampled only once. Therefore private supplies that appeared to be of a high quality, may have given poorer results if sampled on more than one occasion. Likewise, those indicating poor water quality may have yielded a higher water quality if sampled on more than one occasion. In none of the samples was *E. coli* O157 detected, however its isolation from private water supplies has been reported in other studies. In one study in the Netherlands *E. coli* O157 was isolated from 2.7% of private water supplies (Schets *et al* 2005). It is also widely recognised that private water supplies have been responsible for outbreaks of *E. coli* O157 both in Scotland and elsewhere (Licence *et al* 2001 and Smith *et al* 2006). This study did not investigate the presence of other waterborne pathogens, in particular *Cryptosporidium* and enteroviruses, which are also important potential contaminants of private water supplies.

There was a significant association between the detection of coliforms and both *E. coli* and Enterococci. Such a relationship could be expected and whilst demonstrating the potential of coliforms to act as an in indicator of water quality, on its own it does not provide the complete picture of water quality which would be only available through also testing for the presence and enumeration of other pathogens.

It is interesting to note that there appeared to be no overall difference in the water quality of samples from cases and controls (Tables 53 to 55). As this was the first case control study to sample private water supplies from cases and controls, it was not possible to compare the results to other studies. However, this finding is comparable to the findings of a prospective study of rural drinking water quality and acute gastrointestinal illness. The study included a self-report diary kept for 28 days with a check list of acute gastrointestinal symptoms and testing of two water samples taken from the household two weeks apart during the 28 day observation period. While 8.2% reported one or more episodes of acute gastrointestinal illness, no statistically significant association was observed between total coliform or *E.coli* counts and self-reported acute gastrointestinal illness (Strauss *et al* 2001)

Campylobacter were detected in a total of three samples: two from private water supplies from cases and the third from a control where the source was coded as both private water and mains supply, with the sample taken from the private water supply. *Campylobacter* have been demonstrated to form a ‘viable but non-culturable’ (VBNC) state in response to extremes in; pH, moisture content, temperature, nutrient content and salinity. The VBNC state of *Campylobacter* was first described in work investigating the survival of the bacteria in aquatic environments (Rollins and Colwell 1986). In its VBNC state the ability to culture the bacteria is lost even through the bacteria is alive and metabolically active, this is physiologically important state as it allows survival until environmental conditions become favourable for growth and cell division (Jackson *et al* 2009). Work by Moore *et al* (2001) used a molecular detection test using extracted DNA and PCR when testing a range of drinking, recreational and environmental water samples for *Campylobacter*, whilst this

methodology would have also detected *Campylobacter* in a VBNC state, it would have also detected dead cells. When assessing the survival of *Campylobacter* in inoculated mineral water, Guillou *et al* (2008) used passage into embryonated eggs to enable to recovery of cells from a VBNC state. Such techniques were not employed in the testing protocol used in this study, and hence the identification of *Campylobacter* from just three private water supplies may have underestimated the true prevalence, with other supplies potentially containing *Campylobacter* in a viable but non-culturable state, and hence still potentially able to cause infection.

Since the number of *Campylobacter* positive samples was so small, it was not possible to determine whether there was any statistical association between the presence of *Campylobacter* in the sample and being a case or control. Likewise it was not possible to determine if there was any seasonal association with one isolation occurring in each of April, October and December. The low number of *Campylobacter* positive samples in this study may reflect that contamination of private water supplies with *Campylobacter* is an infrequent low level event. However this low level contamination of water supply may be enough to cause infection. As samples were taken an average of 34 days after the onset of illness, the results do not necessarily reflect water quality in the days prior to onset, but rather overall water quality. The presence of *Campylobacter* in the supply could be related to factors such as heavy rainfall especially after a dry spell with run off leading to contamination of the water source, or direct contamination for example through animal faeces entering a well etc as well as the survival of the pathogen in the water and its subsequent successful isolation.

The investigation of the seasonality following the detection of coliforms, *E.coli* and Enterococci was hampered by the relatively low number of private water supply samples tested each month (Appendix Figures 6 to 8), although there was an indication that the detection of these pathogens was less frequent at the start of the year. These results must be viewed with caution due to the small numbers involved. This study did not aim to collect any data on rainfall or other weather parameters. Due to the environment involved it would have been necessary to have such information on much smaller geographical areas than is readily available and to be meaningful would also need to be available for both the time prior to onset and the time of the sample being taken. Although this study did not seek to include weather as a parameter, it has been reported by others to be an influencing factor on private water supply quality. The study of Kay *et al* (2007) of seven large commercial private water supplies found that poor microbiological water quality typically followed periods of heavy rainfall. In a previous study in Aberdeenshire the microbiological failure rates displayed a seasonal trend being greater during the latter half of the year. It was reported that although this observation was likely to be due to a combination of local and regional factors, part of the variability in failure rate was explained by a significant positive relationship with rainfall amount (Reid *et al* 2003). A study of waterborne disease outbreaks in Canada (1975-2001) suggested that warmer temperatures and extreme rainfall were contributing factors to waterborne disease outbreaks (Thomas *et al* 2006). A study of rainfall and outbreaks of drinking water

related disease in England and Wales found evidence that both periods of low rainfall and heavy rain precede many drinking water outbreaks (Nichols *et al* 2009).

There were no significant differences between Category A or B supplies being positive for coliforms*, E. coli* or Enterococci (Tables 57 to 59)*.* Where this information was available, 71 of the supplies were reported as Type B and only five Type A. Whilst every effort was made for the accurate assignment of Type A or B to a supply, this accuracy can not be guaranteed, furthermore the current classification scheme came into force during the study.

Under the Private Water Supplies (Scotland) Regulations 2006, Type A supplies are those providing 10m^3 or more water a day or serving 50 or more persons and supplies to commercial or public activities regardless of size. Under the regulations it is these Type A supplies that are required to meet the water quality standards set by the directive. It could therefore be suggested that the quality of Type A supplies might be greater than that of Type B supplies. However, it must be taken into consideration that where information was available only 7% of supplies tested in this study were type A. Also the new regulations only came into force in July 2006, which was during the study, and it may take a number of years for the regulations to result in an improvement of the quality of such supplies.

For 81% (62/77) of sampled private water supplies information was available from the questionnaire on whether the participant believed the supply was treated or not, with 35% (22) reported to be treated and 65% (40) not treated, the study had no means of verifying these responses. In untreated supplies, coliforms and *E. coli* (Tables 60 & 61) were significantly more likely to be detected than in treated supplies, although there was no significant difference in their enumeration or the detection and enumeration of Enterococci (Table 62)*.* Although these results highlight the improvement of water quality achieved with the treatment of supplies, the fact that among the treated supplies 32%, 9% and 18% were still positive for coliforms*, E. coli* and Enterococci*,* respectively, they equally highlight the need for the treatment mechanisms to be properly maintained, for example filters being replaced at appropriate intervals**.** These results also highlight that it is important that a treatment system installed to a private water supply does not provide a false sense of security about the quality of the water.

The provision of the water testing results to the participants was accompanied by information about improvement of the supply. Under the Private Water Supplies (Grants) (Scotland) Regulations 2006, grants of up to £800 are available from local authorities for the improvement of private water supplies. The study did not seek to follow up private water supplies, to ascertain, if any improvements had been made to the supply as a consequence of the water testing results obtained by taking part in this study or if any participants had applied for grants for the improvement of supplies. As a consequence of participation in the water testing component of the study, an increased awareness was probably achieved among those on private water supplies of

the issues of water quality and the potential for microbial contamination of their drinking water.

7.16 MLST study component

The *Campylobacter* case control study was conducted at the same time as a project S14006 which carried out molecular typing all *Campylobacter* isolates in Scotland over a period of 14 months using MLST. In Grampian the MLST study was extended to cover the whole duration of the case control study. MLST results comprising species, clonal complex (CC) and sequence type (ST) were available and linked to case questionnaire data for 700 (88.7%) cases. To the best of our knowledge this is the first time that MLST data have been available to be included in a case control study. This combination of molecular typing and epidemiological information is unique and provides a valuable resource for understanding *Campylobacter* epidemiology in Scotland.

Two parallel FSAS funded studies - the MLST project (S14006) and the temporal and geographic variation project (S14004) explored in more detail the source attribution of different *Campylobacter* sequence types. Their analysis showed that all host species and food sources generally contained very high levels of ST diversity. Most of the STs found in potential infection sources also occurred in clinical isolates, and this allowed human *Campylobacter* cases to be attributed to these sources. In the MLST project, the most common ST in clinical isolates (ST257) was also most commonly found in retail chicken. Approximately three-quarters of clinical isolates could be attributed to each of six potential sources: less than 1% to pigs, 5-6% to wild birds, 12-15% each to cattle, sheep and companion animals, and just over 30% to retail chicken. When companion animals were excluded the attributions were similar: less than 1% to pigs, 7-8% to wild birds, 15-18% each to cattle and sheep, and 35-36% to retail chicken. The MLST study clearly identified retail chicken as the single largest source of clinical *Campylobacter* infection in Scotland, consistent with *Campylobacter* prevalence and bacterial loads in broiler chickens and with other case control studies. The MLST study also identified farm ruminants as sources, for which infection routes are uncertain and since confirming studies are rare, this was the most controversial finding of the study.

Within the full Scottish dataset for the MLST study, 90.2% of clinical isolates were *C. jejuni* and 9.6% *C. coli* (Forbes 2009). Similar results were observed for isolates from this case control study with 94.1% and 5.4%, respectively, although interestingly the proportion of *C. coli* was slightly lower in the case control study.

Across the 12 mainland NHS boards in the MLST study, the most frequently reported sequence type among the clinical isolates was ST 257, closely followed by ST 21 and ST 45. In the case control study the most commonly identified was ST 21 (12.1%) followed by ST 257 (7.7%), ST 48 (6.3%) and ST 45 (6.0%). The MLST study reported ST 257 to be common among isolates from retail chicken.

For seven of the nine households with more than one case participating in the study, information was available for the MLST profile of all cases resident in the household. In five of these seven households the isolates were the same ST and CC. Interestingly in all five of these households the isolates were of CC 21 which was the most prevalent of the clonal complexes in the study. In the other two households the two cases in the house were of different MLST types. This does not preclude the possibility that the household members acquired their infections from the same source. It is known even if cases acquire their infection from the same source their *Campylobacter* isolates may be of different MLST types. During the study period a large outbreak of *Campylobacter* was investigated in which chicken liver pate was identified as the vehicle of infection. The isolates from cases in this outbreak were typed as part of the MLST project and four different sequence types identified among these cases. This involvement of more than one type (using a number of typing methods) has also been reported by others (Clark *et al* 2003, Frost *et al* 2002). While other *Campylobacter* outbreaks have reported the involvement of just one type (Roels *et al* 1998, Evans *et al* 1998, Engberg *et al* 1998).

The study showed that there was a significant association between the month of illness and CC. It would appear for example, there were more cases of ST45 in May/June than expected and fewer in November/December, which is consistent with the findings of the MLST project.

A number of associations between exposure characteristics and either strain type or source attribution were identified. The most striking positive pattern was the highly distinctive strain composition of cases with an overnight stay abroad, and the greater diversity of strains in this group.

The next strongest positive pattern was the association of ruminant-attributed strains with cases with farm animal contact and those who had a private water supply. In parallel, there was no evidence of differences in either strain composition or host attribution associated with the consumption of red meat prepared at home. These patterns suggest that cases with ruminant attributed strains reflect transmission by direct contact and not foodborne routes, although limited information was collected on consumption of red meat and firm conclusions would require more evidence.

Surprisingly there was no difference in either strain composition or host attribution values associated with chicken consumption through chicken eaten outside the home, chicken prepared at home or both. This is surprising because this study and a number of other studies (Effler *et al* 2001, Rodrigues *et al* 2000, Michaud *et al* 2004, Unicomb *et al* 2008, Friedman *et al* 2004, Eberhart-Phillips *et al* 1997, Evans *et al* 2003, Baker & McLean 2005) have identified chicken eaten out as a risk factor for *Campylobacter* infection. This result could be due to a number of reasons. Firstly there may have been insufficient data. The study was not specifically designed to address the issue of a link between *Campylobacter* infection and chicken consumption. More specific investigation of this topic may have been required as the ubiquity of

chicken consumption may have influenced the findings, or the molecular host attribution models used here were not correctly parameterised. However poor molecular attribution is unlikely to be significant in this case as no strong correlation with strain type was observed even through there was an association with farm animal contact and strain type (CC/ST) and strongly with ruminant-attributed strains.

In the water testing component of the study, *Campylobacter* was isolated from three private water supplies. One of these private water supplies belonged to a control and no MLST typing was available for this isolate. The other isolates were detected in two private water supplies belonging to cases, in both instances the clinical isolates from the cases were typed as ST 45 and CC 45. In both instances the *Campylobacter* isolates from the private water supplies, were of different sequence types and clonal complexes (ST 1614 CC 828 and ST 1286). The finding of different molecular types in the private water supplies compared to the cases in not too surprising. Firstly, although private water supplies were identified as a significant risk factor for *Campylobacter* infection, this does not prove that these individual cases acquired their infection from their private water supply. Secondly, the water samples were taken some time after the cases were infected, and it is possible that the water supplies have been contaminated with *Campylobacter* on a number of occasions, and that at any one time multiple strains of *Campylobacter* were present, but only one of these was isolated for the molecular typing. No strong pattern of host attribution for ST45 was reported in the MLST project therefore it was not possible to speculate in this study as to likely sources of infection for these cases from which this particular ST was isolated. The isolates detected from the water supplies were ST 1268 and ST 1614. ST 1286 has a strong association with pigs (0.838) and ST 1614 has likely attribution to pigs (0.558) and sheep (0.446). The latter isolate was CC 828 which in the MLST study had shown an over-attribution to pigs, that stems from virtually all *C. coli* ST's being members of CC 828.

7.17 Further research

The findings from this work have identified a number of areas for future research to increase understanding of the epidemiology of *Campylobacter* and the most effective control strategies.

Among avenues of potential future research is that of possible protective factors for *Campylobacter* infection. The study suggests that the consumption of fresh fruit, vegetables and salads may be associated with a reduced risk of *Campylobacter* infection. As this study was not designed to investigate any association with fruit or vegetables, the questions were very limited. More detailed investigations would be required to establish the quantities and types involved and to elucidate if this apparent protection is a result of those who report consumption of fruit, salads or vegetables do so at the expense of consuming poultry or other foods. Any future research in this area could consider not only *Campylobacter* but other gastrointestinal pathogens and seek to establish the biological mechanisms for any protection.

Future research could also consider attitudes towards the risks associated with private water supplies and the most effective means of encouraging those with private water supplies to take measures to mitigate these risks.

Further research could also consider the factors that are inherently linked to private water supplies including rurality and their impact on *Campylobacter* infection, to some extent is this being covered in a separate Food Standards Agency (Scotland) funded study investigating the temporal and geographic variation in *Campylobacter* infection in Scotland (S14004).

7.18 Final Conclusions

The study has clearly demonstrated the for the first time in Scotland that the consumption of water from private water supplies is a risk factor for *Campylobacter* infection in Aberdeenshire and Aberdeen City and has highlighted the significant difference in the microbial quality of water from private water supplies compared to mains supplies. It is important that these findings inform any future actions taken to reduce the risk associated with private water supplies. The outcomes should also be used to educate those who use private water supplies about the potential associated risks and actions that could be taken to address these risks.

The study also identified other risk factors for *Campylobacter* infection including travel aboard, and has had the unique opportunity to investigate the epidemiology of *Campylobacter* infection with the inclusion of molecular typing data. A number of associations between exposure characteristics and either strain type or source attribution were identified in particular for travel abroad, contact with farm animals and having a private water supply.

8. SCIENTIFIC PUBLICATIONS

8.1 Papers in preparation

Private water supplies as a risk factor for *Campylobacter* infection

Microbial quality of private water supplies

Clinical presentation of *Campylobacter* infection and factors influencing hospitalisation

Differences in MLST strains of *Campylobacter* infection from those who have travelled abroad compared to indigenous cases

8.2 Presentations at Scientific and stakeholder meetings

Private water supplies as a risk factor for *Campylobacter* infection in Aberdeen City and Aberdeenshire. Food Standards Agency Scotland, Dissemination of *Campylobacter* research day. Edinburgh 2009.
http://www.food.gov.uk/news/newsarchive/2009/dec/campylobacter

21st International Food Microbiology Symposium, September 2008
Case control study of private water supplies as a risk factor for *Campylobacter* infection in Aberdeen City and Aberdeenshire. A. Smith-Palmer, J. Cowden, S. Donaldson, H. Howie, J. Horne, J. McElhiney, I. Ogden, N. Strachan, D. Cooper, D. Reid, S. O'Brien

HPA Health Protection Conference. Warwick, September 14-15 2010
Case control study of private water supplies as a risk factor for *Campylobacter* infection in Aberdeen City and Aberdeenshire. Smith-Palmer A., Cowden J., Howie H., Leith J., Strachan N & Ogden I.

HPA Health Protection Conference. Warwick, September 14-15 2010
Microbial quality of private water supplies in Aberdeenshire and Aberdeen City. Smith-Palmer A., Cowden J., Howie H., Leith J., Strachan N & Ogden I.

9. REFERENCES

Aberdeenshire Council – Private Water Supplies. www.aberdeenshire.gov.uk/environmental/water. Accessed 10th January 2008

Acheson D. & Allos B.M. (2001) *Campylobacter jejuni* infections: update on Emerging Issues and Trends. *Clinical Infectious Diseases* **32** (8) 1201-1209

Adak G.K., Long S.M. & O'Brien S.J. (2002) Trends in indigenous foodborne diseases and deaths, England and Wales: 1992 to 2000. *Gut* **51,** 832-841

Adak G.K., Cowden J.M., Nicholas S. & Evans H.S. (1995) The Public Health Laboratory Service national case-control study of primary indigenous sporadic cases of campylobacter infection. *Epidemiology and Infection* **115,** 15-22

Alary M. & Nadeau D. (1990) An outbreak of Campylobacter enteritis associated with a community water supply. *Canadian Journal of Public Health* **81** (4) 268-271

Allos B.M (1997) Association between *Campylobacter* infection and Guillain-Barré syndrome. *Journal of Infectious Disease* **176** (Suppl 2) S125-128

Altekruse S.F., Stern N.J., Fields P.I. & Swerdlow D.L. (1999) *Campylobacter jejuni* – An Emerging Foodborne Pathogen. *Emerging Infectious Diseases* **5** (1) 28-36

ARLEQUIN website: cmpg.unibe.ch/software/arlequin3/

Baker M., Wilson N and Edwards R. (2007) *Campylobacter* infection and chicken: an update on New Zealand's largest 'common source outbreak'. *The New Zealand Medical Journal* **120** (1261)

Barrell R.A.E., Hunter P.R. & Nichols G. (2000) Microbiological standards for water and their relationship to health risk. *Communicable Disease and Public Health* **3** (1) 8-13

Benjamini Y. & Hochberg Y (1995) Controlling the false discovery rate – a practical and powerful approach to multiple testing. *J R Stat Soc Ser B-Methodology* **57:** 289-300

Blaser M.J. (1997) Epidemiologic and clinical features of *Campylobacter jejuni* infections. *Journal of Infectious Disease* **176,** S103-105

Butzler J-P., Dekeyser P., Detrain M. & Dehaen F. (1973) Related vibrio in stools. *Journal of Paediatrics* **82,** 493-495

Carrique-Mas J., Andersson Y., Hjertqvist M., Svensson A., Torner A. & Giesecke J. (2005) Risk factors for domestic sporadic campylobacteriosis among young children in Sweden. *Scandinavian Journal of Infectious Disease* **37** (2) 101-110

Carstairs Report.
http://www.sphsu.mrc.ac.uk/files/File/library/other%20reports/Carstairs_report.pdf

Clark G.C., Price L., Ahmed R., Woodward D.L., Melito P.L., Rodgers F.G., Jamieson F., Ciebin B., Li Aimin L. & Ellis A. (2003) Characterization of Waterborne outbreak-associated *Campylobacter jejuni,* Walkerton, Ontario. *Emerging Infectious Diseases* **9** (10) 1232-1241

Coker A.O., Isokephi R.D., Thomas B.N., Amisu K.O. & Obi C.L. (2002) Human campylobacteriosis in Developing Countries. *Emerging Infectious Diseases* **8** (3) 237-243

Cowden J. & Smith-Palmer A. (2006) General outbreaks of infectious intestinal disease reported to HPS in the fourth quarter of 2005. *HPS Weekly Report* **40** (13)

Damborg P., Olsen K.E.P., Nielsen E.M and Guardabassi L. (2004) Occurrence of *Campylobacter jejuni* in pets living with human patients infected with *C.jejuni. Journal of Clinical Microbiology* **42** (3) 1363-1364

De Wit M.A.S., Koopmans M.P.G., Kortbeek L.M., van Leeuwen N.J., Vinjé J & van Duynhoven Y.T.H.P (2001) Etiology of Gastroenteritis in Sentinel General Practices in The Netherlands. *Clinical Infectious Diseases* **33,** 280-288.

Dingle K.E., Colles F.M., Ure R., Wagenaar J.A., Duim B., Bolton F.J., Fox A.J., Wareing D.R.A and Maiden M.C.J. (2002) Molecular characterization of *Campylobacter jejuni* clones: A basis for epidemiologic investigation. *Emerging Infectious Diseases* **8** (9) 949-955

Drinking Water Quality in Scotland 2006 (2007) Annual report by the drinking water quality regulator.

Drinking Water Quality in Scotland 2005 (2006) Annual report by the drinking water quality regulator
www.scotland.gov.uk/publications/2006/07/31092205/4

Drinking Water Quality in Scotland 2002 (2003) Annual report by the drinking water quality regulator.
www.Scotland.gov.uk/publications/2003/10/18341/27846

Eberhart-Phillips J., Walker N., Garrett N., Bell D., Sinclair D., Rainger W. & Bates M. (1997) Campylobacteriosis in New Zealand: results of a case control study. *Journal of Epidemiology and Community Health* **51,** 686-691.

Effler P., Ieong M-C., Kimura A., Nakata M., Burr R., Cremer E. and Slutsker L. (2001) Sporadic *Campylobacter jejuni* infections in Hawaii: Associations with prior antibiotic use and Commercially prepared chicken. *The journal of Infectious Diseases* **183,** 1152-1155

Engberg J., Gerner-Smidt P., Scheutz F., Moller Nielsen E., On S.L., Molbak K. (1998) Water-borne *Campylobacter jejuni* infection in a Danish town – a 6 week continuous source outbreak. *Clinical Microbiological Infection* **4 (11)** 648-656

Ethelberg S., Simonsen J., Gerner-Smidt P., Olsen K.E.P. & Mølbak K. (2005) Spatial Distribution and Registry-based Case-Control Analysis of *Campylobacter* infections in Denmark, 1991-2001. *American Journal of Epidemiology* **162** (10) 1008-1015.

Ethelberg S., Olsen K.E.P., Gerner-Smidt P. & Mølbak K. (2004) Household outbreaks among culture-confirmed cases of bacterial gastrointestinal disease. *American Journal of Epidemiology* **159** (4) 406-412

Evans M.R., Ribeiro C.D. & Salmon R.L. (2003) Hazards of healthy living: Bottled water and salad vegetables as risk factors for Campylobacter infection. *Emerging Infectious Diseases* **9** (10) 1219-1225

Evans M., Lane W., Frost J.A. & Nylen G. (1998) A *Campylobacter* outbreak associated with stir-fried food. *Epidemiology and Infection* **121,** 275-279

Excoffier L., Laval G. & Schneider S. (2005) Arlequin (version 3.0): An integrated software package for population genetics data analysis. *Evolutionary Bioinformatics Online* **1**: 47-50

Fisher's Exact Test Excel add-in website:
www.obertfamily.com/software/fisherexact.html

Fisher's Exact Test online calculator:
www.graphpad.com/quickcalcs/contingency1.cfm.

Forbes K. (2009) The molecular epidemiology of Scottish *Campylobacter* isolates from human cases of infection using Multilocus Sequence Typing (MLST). Food Standards Agency Scotland contract S14006.
http://www.food.gov.uk/multimedia/pdfs/publication/fullreportcamps.pdf

Friedman C.R., Hoekstra R.M., Sammuel M., Marcus R., Bender J., Shiferaw B., Reddy S., Ahuja S.D., Helfrick D.L., Hardnett F., Carter M., Anderson B. & Tauxe R.V; Emerging infectious program FoodNet working group. (2004) Risk factors for sporadic Campylobacter infection in the United States: A case-control study in FoodNet sites. *Clinical Infectious Diseases* **38** (Suppl 3) S285-296

Frost J.A., Gillespie I.A. & O'Brien S.J. (2002) Public health implications of *Campylobacter* outbreaks in England and Wales, 1995-9: epidemiological and microbiological investigations. *Epidemiology and Infection* **128** (2) 111-118

Frost J.A., Kramer J.M. & Gillenders S.A. (1999) Phage typing of *Campylobacter jejuni* and *Campylobacter coli* and its use as an adjunct to serotyping. *Epidemiology and Infection* **123,** 47-55

Gallay A., Bousquet V., Siret V., Prouzet-Mauléon V., de Valk H., Vaillant V., Simon F., Le Strat Y., Mégraud F. & Deseclos J-C. (2008) Risk factors for acquiring sporadic *Campylobacter* infection in France: Results from a national case-control study. *Journal of Infectious Disease* **197,** 1477-1484

General Register Office for Scotland. Mid-2006 Population Estimates Scotland. Population estimates by sex, age and administrative area. www.gro-Scotland.gov.uk/files1/stats/mid-2006-population-estimates-scotland/mid-2006-population-estimates-scotland.pdf

Gillespie I.A., O'Brien S.J., Frost J.A., Tam C., Tompkins D., Neal K.R., Syed Q., Farthing M.J.G. & the Campylobacter Sentinel Surveillance Scheme Collaborators (2006). Investigating vomiting and/or bloody diarrhoea in *Campylobacter jejuni* infection. *Journal of Medical Microbiology* **55,** 741-746

Gillespie I.A and The *Campylobacter* Sentinel Surveillance Scheme Collaborators (2003) Point source outbreaks of *Campylobacter jejuni* infection – are they more common than we think and what might cause them? *Epidemiology and Infection* **130,** 367-375

Gillespie I.A., O'Brien S.J., Frost J.A., Adak G.K., Horby P., Swan A.V., Painter M.J., Neal K.R., & the Campylobacter Sentinel Surveillance Scheme Collaborators. (2002) A case-case comparison for *Campylobacter jejuni* and *Campylobacter coli* infection: a tool for generating hypotheses. *Emerging Infectious Diseases* **8** (9) 937-942

Grajewski B.A, Kusek J.W. & Gelfand H.M. (1985) Development of a bacteriophage typing system for *Campylobacter jejuni* and *Campylobacter coli. Journal of Clinical Microbiology* **22,** 13-18

Green C.G., Krause D. & Wylie J. (2006) Spatial analysis of Campylobacter infection in the Canadian province of Manitoba. *International Journal of Health Geographics* **5** (2)

Green MS. (1992) The male predominance in the incidence of infectious diseases in children: a postulated explanation for disparities in the literature. *International Journal of Epidemiology* **21,** 381-386.

Greenwood Z., Black J., Weld L., O'Brien D., Leder K., Von Sonnenburg F., Pandey P., Schwartz E., Connor B.A., Brown G., Freedman D.O. & Torresi J. (2008) Gastrointestinal infection among international travellers globally. *Journal of Travel Medicine* **15** (4) 221-228

Guilou S., Leguerinel I., Garrec N., Renard M.A., Cappelier J.M. & Federighi (2008) Survival of *Campylobacter jejuni* in mineral bottled water according to difference in mineral content: application of the weibull model. *Water Research* **42** (8-9) 2213-2219

Gűrtler M., Alter T., Kasimir S. & Fehlaber K. (2005) The importance of *Campylobacter coli* in human campylobacteriosis: prevalence and genetic characterization. *Epidemiology and Infection* **133,** 1081-1087

Health Protection Agency Website
www.hpa.org.uk/infections/topics_az/campy

Hanninen M.L., Haajanen H., Pummi T., Wermundsen K., Katila M.L., Sarkkinen H., Miettinen I., Rautelin H. (2003) Detection and typing of *Campylobacter jejuni* and *Campylobacter coli* and analysis of indicator organisms in three waterborne outbreaks in Finland. *Applied Environmental Microbiology* **69 (3)** 1391-1396

Hannu T., Mattila L., Rautelin H., Pelkonen P., Lahdenne P., Siitonen A. & Leirisalo-Repo M. (2002) *Campylobacter*-triggered reactive arthritis: a population-based study. *Rheumatology* **41,** 312-318

Hopkins K.L., Desai M., Frost J.A., Stanley J. & Logan J.M. (2004) Fluorescent amplified fragment length polymorphism genotyping of *Campylobacter jejuni* and *Campylobacter coli* strains and its relationship with host specificity, serotyping, and phage typing. *Journal of Clinical Microbiology* **42** (1) 229-235

Hughes R.A. & Cornblath D.R. (2005) Guillain-Barre syndrome. *Lancet* **366** (9497) 1653-1666

Humphrey T., O,Brien S. & Madsen M. (2007) Campylobacters as zoonotic pathogens: A food production perspective. *International Journal of Food Microbiology* **117,** 237-257

Hunter P.R. & Gaston M.A. (1988) Numerical index of the discriminatory ability of typing systems: an application of Simpson's index of diversity. *Journal of Clinical Microbiology* **26,** 2465-2466

Jackson D.N., Davis B., Tirado S.M., Duggal M., van Frankenhuyzen J.K., Deaville D., Wijesinghe M.A.K., Tessaro M. & Trevours J.T. (2009) Survival mechanisms and culturability of *Campylobacter jejuni* under stress conditions. *Antonic van Leeuwenhoek* **96,** 377-394

Jørgensen F., Bailey R., Williams S., Henderson P., Wareing D.R.A., Bolton F.J., Frost J.A., Ward L. & Humphrey T.J. (2002) Prevalence and numbers of *Salmonella* and *Campylobacter* spp. on raw whole chickens in relation to sampling methods. *International Journal of Food Microbiology* **76,** 151-164

Kapperud G., Espeland G., Wahl E., Walde A., Herikstad H., Gustavsen S., Tveit I., Natas O., Bevanger L. & Digranes, A. (2003) Factors associated with increased and decreased risk of Campylobacter infection: a prospective case-control study in Norway. *American Journal of Epidemiology* **158** (3) 234-242

Kay D., Watkins J., Francis C.A., Wyn-Jones A.P., Stapleton C.M., Fewtrell L., Wyre M.D. & Drury D. (2007) The microbiological quality of seven large commercial private water supplies in the United Kingdom. *Journal of Water Health* **5** (4) 523-538.

Kuusi M., Nuorti J.P., Hanninen M.L., Koskela M., Jussila V., Kela E., Miettinen I. & Ruutu P. (2005) A large outbreak of campylobacteriosis associated with a municipal water supply in Finland. *Epidemiology and Infection* **133** (4) 593-601

Licence K., Oates K.R., Synge B.A & Reid T.M. (2001) An outbreak of *E.coli* O157 infection with evidence of spread from animals to man through contamination of a private water supply. *Epidemiology and Infection* **126** (1) 135-138

Maiden M.C., Bygraves J.A., Feil E., Morelli G., Russell J.E., Urwin Q., Zhang J., Zhou K., Zurth D.A., Caugant I.M., Feavers M., Achtman & Spratt B.G. (1988) Multilocus sequence typing: a portable approach to the identification of clones within populations of pathogenic microorganisms. *Proc. Natl. Acad.Sci.USA* **95,** 3140-3145

Manning G., Dowson C.G., Bagnall M.C., Ahmed I.H., West M. & Newell D.G (2003) Multilocus sequence typing for comparison of veterinary and human isolates of *Campylobacter jejuni. Applied and Environmental Microbiology* **69** (11) 6370-6379

Martin S., Penttinen P., Hedin G., Ljungström M., Allestam G., Andersson Y. & Giesecke, J. (2006) A case-cohort study to investigate concomitant waterborne outbreaks of *Campylobacter* and gastroenteritis in Söderhamn, Sweden, 2002-3. *Journal of Water Health* **4** (4) 417-424

Meinermann R.J., Helsel L.O., Fields P.I. & Hiett K.L. (1997) Discrimination of *Campylobacter jejuni* isolates by *fla* gene sequencing. *Journal of Clinical Microbiology* **35,** 2810-2814

Melby K., Gondrosen B., Gregusson S., Ribe H. & Dahl, O.P. (1991) Waterborne campylobacteriosis in northern Norway. *International Journal of Food Microbiology* **12 (2-3)** 151-156.

Meldrum R.J. & Wilson I.G. (2007) *Salmonella* and *Campylobacter* in United Kingdom retail raw chicken in 2005. *Journal of Food Protection* **70** (8) 1937-1939

Meldrum R.J., Smith R.M. & Wilson I.G. (2006) Three year surveillance program examining the prevalence of *Campylobacter* and *Salmonella* in whole retail raw chicken. *Journal of Food Protection* **69** (4) 928-31

Merritt A., Miles R & Bates J. (1999) An outbreaks of *Campylobacter* enteritis on an island resort, north Queensland. *Communicable Disease Intelligence* **23,** 215-219

Michaud S., Ménard S. & Arbeit R.D. (2004) Campylobacteriosis, Eastern Townships, Québec. *Emerging Infectious Diseases* **10** (10) 1844-1847

Moore J.E., Corconan D., Dooley J.S.G., Fanning S., Lucey B., Matsuda M., McDowell D.A., Mégraud F., Millar B.C., O'Mahony R., O'Riordan L., O'Rourke M., Rao J.R., Rooney P.J., Sails A. & Whyte P. (2005) Campylobacter. A review article. *Veterinary Research.* **36,** 351-382

Moore J., Caldwell P. & Millar B. (2001) Molecular detection of *Campylobacter* spp. in drinking, recreational and environmental water supplies. *International journal of Hygiene and Environmental Health* **204** (2-3) 185-189

Nachamkin I., Bohachick K & Patton C.M. (1993) Flagellin gene typing of *Campylobacter jejuni* by restriction fragment length polymorphism analysis. *Journal of Clinical Microbiology* **31,** 1531-1536

Neal K.R. & Slack R.C. (1997) Diabetes mellitus, anti-secretory drugs and other risk factors for campylobacter gastro-enteritis in adults: a case-control study. *Epidemiology and Infection* **119** (3) 307-311

Neimann J., Engberg J., Molbak K. & Wegener H.C. (2003) A case control study of risk factors for sporadic campylobacter infection in Denmark. *Epidemiology and Infection* **130** (3) 353-366

Nichols G., Lane C., Asgari N., Verlander NQ. & Charlett A. (2009) Rainfall and outbreaks of drinking water related disease in England and Wales. *Journal of Water Health* **7** (1) 1-8

Nygård K., Andersson Y., Røttingen J.A., Svensson A., Lindbäck J., Kistemann T. & Giesecke J. (2004) Association between environmental risk factors and *Campylobacter* infections in Sweden. *Epidemiology and Infection* **132,** 317-325

O'Reilly C.E., Bowen A.B., Perez N.E., Sarisky J.P., Shepherd C.A., Miller M.D., Hubbard B.C., Herring M., Buchanan S.D., Fitzgerald C.C., Hill V., Arrowood M.J., Xiao L.M., Hoekstra R.M., Mintz E.D. & Lynch M.F., Outbreak working group (2007). A waterborne outbreak of gastroenteritis with multiple etiologies among resort island visitors and residents: Ohio 2004. *Clinical Infectious Disease.* **44** (4) 506-512

Overell J.R. & Willison H. J. (2005) Recent developments in Miller Fisher syndrome and related disorders. *Current opinion in Neurology* **18** (5) 562-566

Pebody R.G., Ryan M.J. & Wall P.G. (1997) Outbreaks of *Campylobacter* infection: rare event for a common pathogen. *Commun Dis Rep CDR Rev* **7** (3) R33-37

Penner J.L. & Hennessy J.N. (1980) Passive hemagglutination technique for serotyping *Campylobacter fetus* subsp *jejuni* on the basis of soluble heat-stable antigens. *Journal of Clinical Microbiology* **12** 732-737

Poptools website: www.cse.csiro.au/poptools/

Potter R.C., Kaneene J.B. & Hall W.N. (2003) Risk factors for sporadic *Campylobacter jejuni* infections in rural Michigan: A prospective case-control study. *American Journal of Public Health* **93** (12) 2118-2123

Q-VALUE website: www.genomics.princeton.edu/storeylab/qvalue/index.html.

Redman C.A., MacLennan A., Wilson E. & Walker E. (2006) Diarrhoea and respiratory symptoms among travellers to Asia, Africa and South and Central America from Scotland. *Journal of Travel Medicine* **13** (4) 203-211

Reid D.C., Edwards A.C., Cooper D., Wilson E. & Mcgaw, B.A. (2003) The quality of drinking water from private water supplies in Aberdeenshire, UK. *Water Research* **37** (2) 245-254

Richardson G., Thomas D.R., Smith R.M., Nehaul L., Ribeiro C.D., Brown A.G. & Salmon, R.L. (2007) A community outbreak of *Campylobacter jejuni* infection from a chlorinated public water supply. *Epidemiology and Infection* **135** (7) 1151-1158

Roberts J.A., Cumberland P., Sockett P.N., Wheeler J., Robrigues L.C., Sethi D. & Roberick P.J. on behalf of the IID Executive (2003) The study of infectious intestinal disease in England: socio-economic impact. *Epidemiology and Infection* **130,** 1-11

Rodrigues L.C., Cowden J.M., Wheeler J.C., Sethi D., Wall P.G., Cumberland P., Tompkins D.S., Hudson M.J., Roberts J.A. & Roderick P.J. (2001) The study of infectious intestinal disease in England: risk factors for cases of infectious intestinal disease with *Campylobacter jejuni* infection. *Epidemiology and Infection* **127,** 185-193

Rodríguez G.L.A., Ruigómez A. & Panés J. (2007) Use of acid-suppressing drugs and the risk of bacterial gastroenteritis. *Clinicial Gastroenterology and Hepatology* **5** (12) 1418-1423

Roels T.H., Wickus B., Kazmierczak J.J., Nicholson M.A., Kurzynski T.A. & Davis J.P. (1998) A foodborne outbreak of *Campylobacter jejuni* (O:33) infection associated with tuna salad: a rare strain in an unusual vehicle. *Epidemiology and Infection* **121,** 281-287

Rollins D.M. & Colwell R.R (1986) Viable but nonculturable stage of *Campylobacter jejuni* and its role in survival in the natural aquatic environment. *Applied and Environmental Microbiology* **52 (3)** 531-538

Royal Mail Postal Address Book, Scotland 1 & 2 (2001).Royal Mail, Address Management Products, Edinburgh.

Rutter M., Nichols G.L., Swan A. & De Louvois J. (2000) A survey of the microbiological quality of private water supplies in England. *Epidemiology and Infection* **124,** 417-425

Saeed A.M., Harris N.V. & DiGiacomo R.F. (1993) The role of exposure to animals in the etiology of *Campylobacter jejuni/coli* Enteritis. *American Journal of Epidemiology* **137** (1) 108-114

Said B., Wright F., Nichols G.L., Reacher M. & Rutter M. (2003) Outbreaks of infectious disease associated with private drinking water supplies in England and Wales 1970-2000. *Epidemiology and Infection* **130** (3) 469-479

Salfield N.J. & Pugh E.J. (1987) *Campylobacter* enteritis in young children living in households with puppies. *British Medical Journal* **294,** 21-22

Samuel M.C., Vugia D.J., Shallow S., Marcus R., Segler S., McGivern T., Kassenborg H., Reilly K., Kennedy M., Angulo F. & Tauxe R.V; Emerging Infectious Program FoodNet Working Group. (2004) Epidemiology of sporadic *Campylobacter* infection in the United States and declining trend in incidence, FoodNet 1996-1999. *Clinical Infectious Diseases* **38** (Suppl 3) S165-174

Sandberg M., Nygård K., Meldal H., Valle P.S., Kruse H. & Skjerve E. (2006) Incidence trend and risk factors for *Campylobacter* infections in humans in Norway. *BMC Public Health* **6** (179) 1-8

Schets F.M., During M., Italiaander R., Heijnen L., Rutjes S.A., van der Zwaluw W.K. & de Roda Husman A.M. (2005) *Escherichia coli* O157:H7 in drinking water from private water supplies in the Netherlands. *Water Research* **39** (18) 4485-4493

Schönberg-Norio D., Takkinen J., Hänninen M.L., Katila M.L., Kaukoranta S.S., Mattila L. & Rautelin H. (2004) Swimming and *Campylobacter* infections. *Emerging Infectious Diseases* **10** (8) 1474-1477.

Schorr D., Schmid H., Reider H.L., Baumgartner A., Vorkauf H. & Burnens A. (1994) Risk factors for *Campylobacter* enteritis in Switzerland. *Zentralbl hyg Umweltmed* **196** (4) 327-337 (abstract only)

Scottish Government statistics. Population data.
www.gro-scotland.gov.uk/statistics/publications-and-data/population-estimates/1999-and-2000-small-area-population-estimates-scotland.html

Scottish Government statistics. Scottish index of multiple deprivation.
http://www.scotland.gov.uk/Resource/Doc/47251/0027011.pdf

Sheppard S.K., Dallas J.F., Strachan N.J.C., MacRae M., McCarthy N.D., Wilson D.J, Gormley F.J., Falush D., Ogden I.D., Maiden M.C.J & Forbes K.J (2009) *Campylobacter* genotyping to determine the source of human infection. *Clinical Infectious Diseases* **48:** 1072-1078

Simonsen J., Frisch M. & Ethelberg S. (2008) Socieconomic risk factors for bacterial gastrointestinal infections. *Epidemiology* **19** (2) 282-290

Skirrow M.B., Jones D.M., Sutcliff E. & Benjamin J. (1993) *Campylobacter* bacteraemia in England and Wales, 1981-1991. *Epidemiology and Infection* **110,** 567-573

Skirrow M.B. (1977) *Campylobacter* enteritis: a "new" disease. *British Medical Journal* **2:** 9-11

Smith C.L. & Cantor C.R. (2008) Pulsed-field gel electrophoresis of large DNA molecules. *Nature* **319,** 701-702
Smith A., Reacher M., Smerdon W., Adak G.K., Nichols G. & Chalmers R.M. (2006) Outbreaks of waterborne infectious intestinal disease in England and Wales, 1992-2003. *Epidemiology and Infection* **134,** 1141-1149

Smith-Palmer A. & Cowden J. (2009) Overseas outbreaks of infectious intestinal disease identified in Scotland, 2003 to 2007. *Journal of Travel Medicine* **16** (5) 322-327

Smith-Palmer A. & Cowden J. (2003) Demographics and clinical presentation of *Campylobacter* infection in Scotland. 12th International workshop on *Campylobacter, Helicobacter* and related organisms. *International Journal of Medical Microbiology* **293** (Suppl 35) 131

Sopwith W., Ashton M., Frost J.A., Tocque K., O'Brien S., Regan M. & Syed Q. (2003) Enhanced surveillance of *Campylobacter* infection in the North West of England 1997-1999. *Journal of Infection* **46,** 35-45

Stafford R.J., Schluter P., Kirk M., Wilson A., Unicomb L., Ashbolt R., Gregory J. & OzFoodNet Working Group. (2007) A multi-centre prospective case-control study of *Campylobacter* infection in persons aged 5 years and older in Australia. *Epidemiology and Infection* **135,** 978-988

Standard Occupational Classification 2000 Volume 1 – June 2000.
http://www.statistics.gov.uk/methods_quality/ns_sec/downloads/SOC2000.doc

Stanwell-Smith R., Andersson Y. & Levy D.A. (2003) National surveillance systems I: Drinking water and infectious disease – Establishing the links (eds P.R.Hunter, M Waite & E Ronchi) CRC Press, Boca Raton. IN Martin, S. *et al* (2006) A case-cohort study to investigate concomitant waterborne outbreaks of *Campylobacter* and gastroenteritis in Söderhamn, Sweden, 2002-3. *Journal of Water Health* **4** (4) 417-424

Strachan N.J.C., Gormley F.J., Rotariu O., Ogden I.D., Miller G., Dunn G.M., Sheppard S.K., Dallas J.F., Reid T.M.S., Howie H., Maiden M.C.J. & Forbes K.J. (2009) Attribution of *Campylobacter* infections in Northeast Scotland to specific sources by use of multilocus sequence typing. *Journal of Infectious Diseases* **199,** 1205-1208

Strachan N.J.C., Watson R.O., Novik V., Hofreuter D., Ogden I.D. & Galán J.E. (2008) Sexual dimorphism in campylobacteriosis. *Epidemiology and Infection* **136,** 1492-1495

Strauss B., King W., Ley A. & Hoey J.R. (2001) A prospective study of rural drinking water quality and acute gastrointestinal illness. *BMC Public Health* **1:** 8. www.biomedcentral.com/1471-2458/1/8

Stull T.L., LiPuma J.J. & Edlind T.D. (1988) A broad-spectrum probe for molecular epidemiology of bacteria: ribosomal RNA. *Journal of Infectious Disease* **157,** 280-286

Takkinen J., Ammon A., Robstad O. & Breuer T. (2003) European Survey on *Campylobacter* surveillance and diagnosis 2001. *Eurosurveillance* **8** (11) 207-213

Tam C.C., Rodrigues L.C., & O'Brien S.J (2003a) Guillain-Barré Syndrome associated with *Campylobacter jejuni* infection in England, 2000-2001. *Clinical Infectious Diseases* **37,** 307-310

Thomas K.M., Charron D.F., Waltner-Toews D., Schuster C., Maarouf A.R. & Holt J.D. (2006) A role of high impact weather events in waterborne disease outbreaks in Canada, 1975-2001. *International Journal of Environmental Health Research* **16** (3) 167-180

Travel Trends 2006. Data and commentary from the International passenger survey. National statistics (Crown Copyright 2008)
www.statistics.gov.uk/downloads/theme_transport/traveltrends2006.pdf

Unicomb L.E., Dalton C.B., Gilbert G.L., Becker N.G. & Patel M.S. (2008) Age-specific risk factors for sporadic *Campylobacter* infection in regional Australia. *Foodborne Pathogens and Disease* **5** (1) 79-85

van Lieverloo J.H.M., Blokker E.J.M. & Medema G. (2007) Quantitative microbial risk assessment of distributed drinking water using faecal indicator incidence and concentration. *Journal of Water and Health* **05** (Suppl 1) 131-149

V-DICE website: www.hpa-bioinformatics.org.uk/cgi-bin/DICI/DICI.pl

Vos P., Hogers M., Bleeker M, Reijans M., van de Lee T., Hornes M., Frijters A., Pot J., Peleman J., Kuiper M, & *et al* (1995) AFLP: a new technique for DNA fingerprinting. *Nucleic Acids Res* **23,** 4407-4414

Wheeler J.G., Sethi D., Cowden J.M., Wall P.G., Tompkins D.S., Hudson M.J. & Roderick P.J. on behalf of the Infectious Intestinal Disease Executive (1999) *British Medical Journal* **318,** 1046-1050.

Wingstrand A., Neimann J., Engberg J., Nielsen E.M., Gerner-Smidt P., Wegener H.C. & Molbak K. (2006) Fresh chicken as main risk factor for campylobacteriosis in Denmark. *Emerging Infectious Disease* **12** (2) 280-285

Wolfs T.F.W., Duim B., Geelen S.P.M., Rigter A., Thomson-Carter F., Fleer A & Wagenaar J.A. (2001) Neonatal sepsis by *Campylobacter jejuni:* genetically proven transmission from a household puppy. *Clinical Infectious Disease* **32,** E97-E99

APPENDIX A: ADDITIONAL RESULTS TABLES

Appendix Table 1: Uptake rates for cases by study month and year

Study	Month/Year	Reported to NHS Grampian	Participated in study	Uptake rate (%)
Pilot	Aug 05	60	27	45.0
Pilot	Sept 05	49	31	63.3
Pilot	Oct 05	54	34	63.0
Main	Nov 05	51	41	80.4
Main	Dec 05	57	26	45.6
Main	Jan 06	27	21	77.8
Main	Feb 06	37	17	45.9
Main	Mar 06	28	14	50.0
Main	Apr 06	25	16	64.0
Main	May 06	55	29	52.7
Main	Jun 06	70	44	62.9
Main	Jul 06	79	40	50.6
Main	Aug 06	51	42	82.4
Main	Sept 06	49	40	81.6
Main	Oct 06	59	42	71.2
Main	Nov 06	62	31	50.0
Main	Dec 06	15	19	126.7
Main	Jan 07	26	19	73.1
Main	Feb 07	15	11	73.3
Main	Mar 07	29	17	58.6
Main	Apr 07	23	9	39.1
Main	May 07	51	20	39.2
Main	Jun 07	85	42	49.4
Main	July 07	75	45	60.0
Main	Aug 07	76	37	48.7
Main	Sept 07	48	23	47.9
Main	Oct 07	66	52 Received to end of study	78.8
Total		**1322**	**789**	**59.68**

Appendix Table 2: Uptake rate for controls by study month and year

Study	Month/Year	Controls invited to participate	Participated in study	Uptake rate (%)
Pilot	Aug 05	150	28	18.7
Pilot	Sept 05	155	43	27.7
Pilot	Oct 05	0	19	N/A
Main	Nov 05	250	40	16.0
Main	Dec 05	170	72	42.4
Main	Jan 06	185	71	38.4
Main	Feb 06	140	57	40.7
Main	Mar 06	183	62	33.9
Main	Apr 06	195	58	29.7
Main	May 06	250	106	42.4
Main	Jun 06	250	86	34.4
Main	Jul 06	255	94	36.9
Main	Aug 06	250	82	32.8
Main	Sept 06	255	95	37.3
Main	Oct 06	230	74	32.2
Main	Nov 06	240	68	28.3
Main	Dec 06	230	77	33.5
Main	Jan 07	205	59	28.8
Main	Feb 07	185	62	33.5
Main	Mar 07	160	70	43.8
Main	Apr 07	165	43	26.1
Main	May 07	240	91	37.9
Main	Jun 07	260	69	26.5
Main	July 07	275	83	30.2
Main	Aug 07	275	103	37.5
Main	Sept 07	280	71	25.4
Main	Oct 07	280	115 Received to end of study	41.1
Total		**5408**	**1898**	**35.1**

Appendix Table 3: Uptake rates for male controls by age group. (Main study only)

Age group	Invited to participate	Participated	Uptake rate (%)
0-4	138	54	39.1
5-9	89	39	43.8
10-14	90	32	356
15-19	211	40	18.9
20-24	346	37	10.7
25-29	313	35	11.2
30-34	273	48	17.6
35-39	291	55	18.9
40-44	291	91	31.3
45-49	224	73	32.6
50-54	168	77	45.8
55-59	182	95	52.2
60-64	129	66	51.2
65-69	117	67	57.3
70+	141	68	48.2
Total	**3003**	**877**	**29.2**

Appendix Table 4: Uptake rates for female controls by age group (Main study only)

Age group	Invited to participate	Participated	Uptake rate (%)
0-4	95	44	46.3
5-9	70	27	38.6
10-14	84	33	39.3
15-19	171	51	29.8
20-24	276	72	26.1
25-29	243	62	25.5
30-34	200	66	33.0
35-39	200	78	39.0
40-44	210	90	42.9
45-49	174	81	46.6
50-54	157	84	53.5
55-59	159	84	52.8
60-64	121	67	55.4
65-69	109	45	41.3
70+	136	47	34.6
Total	**2405**	**931**	**38.7**

Appendix Table 5: Age, sex and matching frequency of cases and controls

Age band	Males			Females		
	Case	Control	Matching Frequency	Case	Control	Matching Frequency
Under 1	6	6	1.00	0	5	
1-4	29	57	1.97	17	42	2.47
5-9	17	40	2.35	13	28	2.15
10-14	10	34	3.40	7	35	5.00
15-19	16	41	2.56	22	51	2.32
20-24	25	37	1.48	38	74	1.95
25-29	19	35	1.84	27	67	2.48
30-34	21	52	2.48	25	68	2.72
35-39	22	57	2.59	25	83	3.32
40-44	46	94	2.04	37	93	2.51
45-49	31	77	2.48	27	85	3.15
50-54	29	80	2.75	27	87	3.22
55-59	47	101	2.15	42	88	2.10
60-64	33	68	2.06	27	68	2.52
65-69	19	71	3.74	20	46	2.30
70-74	18	40	2.22	8	28	3.50
75-79	14	26	1.86	7	13	1.86
80+	9	10	1.11	9	11	1.22
Total	**411**	**926**	**2.25**	**378**	**972**	**2.57**

Appendix Table 6: Cases and controls in each deprivation category

Deprivation category	Cases Number (%)	Controls Number (%)
1	167 (21.2)	483 (25.5)
2	282 (35.7)	546 (28.8)
3	149 (18.9)	351 (18.5)
4	135 (17.1)	329 (17.3)
5	25 (3.2)	75 (3.9)
6	28 (3.5)	104 (5.5)
7	0	0
Unknown	3 (0.4)	10 (0.5)
Total	**789**	**1898**

Category one is the area of least deprivation, area seven is the area of greatest deprivation.

Appendix Table 7: Distribution of 2001 Deprivation Categories within council areas. (Figures are percentages of council area population)

Council area	Deprivation Category						
	1	2	3	4	5	6	7
Aberdeen city	16	22	14	24	10	14	0
Aberdeenshire	24	34	27	15	0	0	0
Scotland	6	14	22	25	15	11	7

Appendix Table 8: Cases and controls by Scottish Index of Multiple Deprivation (SIMD) Score

SIMD Score	Cases	Controls
	Number (%)	Number (%)
3	7 (0.8)	21 (1.1)
5	48 (6.1)	175 (9.2)
6	39 (4.9)	133 (7.0)
7	102 (12.9)	233 (12.3)
8	130 (16.5)	301 (15.9)
9	57 (7.2)	94 (5.0)
10	44 (5.6)	73 (3.9)
11	17 (2.1)	43 (2.3)
12	27 (3.4)	42 (2.2)
13	18 (2.3)	40 (2.1)
14	37 (4.7)	69 (3.6)
15	6 (0.8)	10 (0.5)
16	25 (3.2)	59 (3.1)
17	36 (4.6)	105 (5.5)
18	34 (4.3)	55 (2.9)
20	23 (2.9)	70 (3.7)
21	44 (5.6)	85 (4.5)
22	4 (0.5)	23 (1.2)
23	17 (2.2)	36 (1.9)
26	17 (2.2)	45 (2.4)
27	22 (2.8)	60 (3.2)
28	4 (0.5)	5 (0.3)
30	4 (0.5)	19 (1.0)
35	0 (0)	9 (0.5)
40	9 (1.1)	22 (1.1)
43	12 (1.5)	37 (1.9)
44	3 (0.4)	26 (1.4)
Unknown	3 (0.4)	8 (0.4)
Total	**789**	**1898**

Occupation Group	Cases	Controls	Total

	Number	percentage	Number	Percentage	
Administrative and secretarial occupations *eg administrative officers, secretaries, receptionists, market research interviewers*	66	8.4	143	7.5	**209**
Associate Professional and Technical Occupations *eg Laboratory technicians, nurses, IT operations technicians, police officers (sergeant & below), sports players, sales representatives, journalists*	67	8.5	160	8.4	**227**
Elementary Occupations *eg farm workers, labourers in building trade, packers, waitresses, bar staff, porters, window cleaners, security guards, shelf fillers*	36	4.6	93	4.9	**129**
Full time Students	29	3.7	58	3.1	**87**
Housewife	41	5.2	116	6.1	**157**
Managers and Senior Officials *eg Corporate managers, production managers, office managers, officers in armed services, farm managers, restaurant managers , police officers (inspectors & above)*	70	8.9	145	7.6	**215**
Part-time	8	1.0	20	1.1	**28**
Personal Service Occupations *eg nursing auxiliaries, air travel assistants, hairdressers, nursery nurses, caretakers, educational assistants*	37	4.7	89	4.7	**126**
Pre School Child	51	6.5	101	5.3	**152**
Process, Plant and Machine Operatives *eg plant & machine operatives, heavy goods vehicle drivers, sewing machinists, scaffolders*,	27	3.4	53	2.8	**80**
Professional Occupations *eg Medical practitioners, veterinarians, engineers, physicists, solicitors, clergy, accountants, teaching professionals*	57	7.2	195	10.2	**252**
Registered Disabled	4	0.5	13	0.7	**17**
Retired	131	16.6	299	15.7	**430**
Sales and Customer Service Occupations *eg Sales & retail assistants, telephone salepersons, market and street traders, check-out operators*	20	2.5	48	2.5	**68**
School Child	57	7.2	187	9.9	**244**
Self Employed	5	0.6	13	0.7	**18**
Skilled Trade Occupations *eg gardeners, electricians, bricklayers, butchers, dressmakers, printers, cooks, motor mechanics*	49	6.2	91	4.8	**140**
Unemployed	13	1.6	40	2.1	**53**
Unknown, missing, not coded	20	2.5	34	1.8	**54**
Total	**789**		**1898**		**2687**

Appendix Table 9: Occupation group of cases and controls

Occupation was a free text field, which was coded by the study researchers to the occupation groups used in the Standard Occupational Classification 2000 Volume 1.

Appendix Table 10: Adults who have a job that involves contact with animals

Occupational contact with animals	Case Number (%)	Control Number (%)
No	662 (96.8)	1583 (97.4)
Yes	10 (1.5)	28 (1.7)
N/K or missing	12 (1.8)	14 (0.9)
Total	684	1625

Based on the occupations provided in the free text field, these were coded by the study researchers to those likely to involve occupational contact with animals.

Appendix Table 11: Adults who have a job that involves contact with raw meat

Occupational contact with raw meat	Case Number (%)	Control Number (%)
No	656 (95.9)	1572 (96.7)
Yes	13 (1.9)	25 (1.5)
Fish	2 (0.3)	8 (0.5)
N/K or missing	13 (1.9)	20 (1.2)
Total	684	1625

Based on the occupations provided in the free text field, these were coded by the study researchers to those likely to involve occupational contact with raw meat.

Appendix Table 12: Completeness of questionnaires for those completing the questionnaire 14 days or less from onset and those completing the questionnaire more than 14 days after onset

Factor	Completed questionnaire within 14 days of onset			Completed questionnaire more than 14 days after onset		
	Yes or No	N/K or Not sure (%)	No answer (%)	Yes or No	N/K or Not sure (%)	No answer (%)
Diarrhoea	538	0	0	228	0	0
Bloody stools	454	79 (14.7)	5 (0.9)	196	31 (13.5)	1 (0.4)
Abdominal pain	527	11 (2.0)	0	223	5 (2.2)	0
Vomiting	530	4 (0.7)	4 (0.7)	225	1 (0.4)	2 (0.8)
Admitted to hospital	535	0	3 (0.6)	228	0	0
Antacids	513	0	25 (4.6)	220	0	8 (3.5)
Antibiotics	513	0	25 (4.6)	217	0	11 (4.8)
Omeprazole etc	527	0	11 (2.0)	225	0	3 (1.3)
Overnight stay outside area	535	0	3 (0.6)	227	0	1 (0.9)
Pets at home	536	0	2 (0.4)	226	0	2 (0.9)
Farm animals	436	0	102 (19.1)	191	0	37 (16.6)
Bottled water	515	18 (3.3)	5 (0.9)	201	22 (9.6)	5 (2.2)
Drinks dispenser	509	13 (2.4)	16 (2.9)	210	8 (3.5)	10 (4.4)
Drinking fountain	483	6 (1.1)	49 (9.1)	198	10 (4.4)	20 (8.7)
Drinking rivers etc	482	4 (0.7)	52 (9.7)	202	4 (1.7)	22 (9.6)
Raw veg washed in tap water	481	14 (2.6)	43 (8.0)	187	29 (12.7)	12 (5.2)
Salads washed in tap water	485	21 (3.9)	32 (5.9)	195	27 (11.8)	6 (2.6)
Fruit washed in tap water	488	20 (3.7)	30 (5.5)	197	22 (9.6)	9 (3.9)
Eating outside the home	527	0	11 (2.0)	213	0	15 (6.6)
Chicken at home	479	30 (5.6)	29 (5.4)	194	13 (5.7)	21 (9.2)
Red meat at home	452	32 (5.9)	54 (10.0)	189	12 (5.2)	27 (11.8)
Cooked meat at home	467	20 (3.7)	51 (9.5)	184	13 (5.7)	31 (13.9)
Salads/raw veg at home	480	11 (2.0)	47 (8.7)	187	17 (7.4)	24 (10.9)
Shellfish at home	452	5 (0.9)	81 (15.1)	184	3 (1.3)	41 (18.3)
Pre-packed sandwiches	452	8 (1.5)	78 (14.5)	188	6 (2.6)	34 (15.3)
Barbecues & picnics	530	0	8 (1.5)	224	0	4 (1.7)
Pre-packed ready to eat foods	514	0	24 (4.5)	217	0	11 (4.8)
Swimming	521	0	17 (3.1)	223	0	5 (2.2)
Canoeing	507	0	31 (5.8)	216	0	12 (5.2)
Sailing	507	0	31 (5.8)	216	0	12 (5.2)
Fishing	510	0	28 (5.2)	217	0	11 (4.8)
Surfing	507	0	31 (5.8)	216	0	12 (5.2)
Diving in sea	509	0	29 (5.4)	216	0	12 (5.2)

Appendix Table 13: Completeness of questionnaires by controls and comparison with all cases.

Factor	All cases			All controls		
	Yes or No	N/K or Not sure (%)	No answer (%)	Yes or No	N/K or Not sure (%)	No answer (%)
Diarrhoea	788	0	1 (0.1)	1876	19 (1.0)	3 (0.2)
Bloody stools	667	115 (14.6)	7 (0.9)	1873	22 (1.1)	3 (0.2)
Abdominal pain	770	18 (2.3)	1 (0.1)	1879	16 (0.8)	3 (0.2)
Vomiting	777	5 (0.6)	7 (0.9)	1890	4 (0.2)	4 (0.2)
Antacids	753	0	36 (4.6)	1842	0	56 (3.0)
Antibiotics	751	0	38 (4.8)	1836	0	62 (3.3)
Omeprazole etc	773	0	16 (2.0)	1850	0	48 (2.5)
Overnight stay outside area	785	0	4 (0.6)	1894	0	4 (0.2)
Pets at home	785	0	4 (0.5)	1890	0	8 (0.4)
Farm animals	646	0	143 (18.1)	1477	0	421 (22.2)
Bottled water	736	43 (5.4)	10 (1.3)	1873	12 (0.6)	13 (0.7)
Drinks dispenser	741	22 (2.8)	26 (3.3)	1841	19 (1.0)	38 (2.0)
Drinking fountain	701	17 (2.2)	71 (9.0)	1739	19 (1.0)	140 (7.4)
Drinking rivers etc	705	8 (1.0)	76 (9.6)	1730	10 (0.5)	158 (8.3)
Raw veg washed in tap water	682	46 (5.8)	61 (7.7)	1781	17 (0.9)	100 (5.3)
Salads washed in tap water	698	50 (6.3)	41 (5.2)	1798	13 (0.7)	87 (4.6)
Fruit washed in tap water	703	44 (5.6)	42 (5.3)	1835	13 (0.7)	50 (2.6)
Eating outside the home	763	0	26 (3.3)	1878	0	20 (1.1)
Chicken at home	693	45 (5.7)	51 (6.5)	1791	5 (0.3)	102 (5.4)
Red meat at home	658	45 (5.7)	86 (10.9)	1767	8 (0.4)	123 (6.5)
Cooked meat at home	669	34 (4.3)	86 (10.9)	1768	6 (0.3)	124 (6.5)
Salads/raw veg at home	684	30 (3.8)	75 (9.5)	1787	7 (0.4)	104 (5.5)
Shellfish at home	653	10 (1.3)	126 (16.0)	1671	4 (0.2)	223 (11.7)
Pre-packed sandwiches	657	16 (2.0)	116 (14.7)	1709	2 (0.1)	187 (9.9)
Barbecues & picnics	775	0	14 (1.8)	1882	0	16 (0.8)
Pre-packed ready to eat foods	751	0	38 (4.8)	1867	0	31 (1.6)
Swimming	766	0	23 (2.9)	1870	0	28 (1.5)
Canoeing	745	0	44 (5.6)	1806	0	92 (5.0)
Sailing	745	0	44 (5.6)	1809	0	89 (4.7)
Fishing	749	0	40 (5.1)	1813	0	85 (4.5)
Surfing	745	0	44 (5.6)	1806	0	92 (4.8)
Diving in sea	747	0	42 (5.3)	1808	0	90 (4.7)

Appendix Table 14: Numbers of others in the same household as the case with similar symptoms. For the 645 cases who report others live in the same household

Number in household with symptoms	Number (%)
0	553 (85.7)
1	69 (10.7)
2	12 (1.9)
3	7 (1.1)
Missing	4 (0.6)
Total	645

Appendix Table 15: Numbers of adult and child cases reporting that at least one other person in the household was ill with similar symptoms, for those that report others live in the same household

Number of others ill	Adult (%)	Child (%)
0	482 (88.9	71 (71.7)
1	50 (9.2)	19 (19.2)
2	7 (1.3)	5 (5.1)
3	3 (0.6)	4 (4.0)
Total	542	99

Appendix Table 16: Two or more laboratory confirmed cases in the same household

Household	Number of confirmed adults and children participating in the study	Days between dates of onset of cases in the same household
1	1 adult, 2 children	All the same date
2	2 children	Both the same date
3	2 children	16 days
4	2 adults	1 day
5	1 adult, 1 child	5 days
6	2 adults	33 days
7	1 adult, 1 child	6 days
8	1 adult, 1 child	64 days
9	2 adults	19 days

Appendix Table 17: Adult, cases and controls taking antacids (eg Rennie, Milk of Magnesia)

Antacids	Case (%)	Control (%)
No	593 (86.7)	1211 (90.1)
Yes	56 (8.2)	98 (7.3)
Missing	35 (5.1)	38 (2.8)
Total	684	1344

Odds ratio = 1.17 (0.82 to 1.67), p = 0.376

There was no significant difference between adult cases and controls in the use of antacids.

Appendix Table 18: Adult, cases and controls taking antibiotics

Antibiotics	Case (%)	Control (%)
No	601 (87.9)	1227 (91.3)
Yes	45 (6.6)	78 (5.8)
Missing	38 (5.6)	39 (2.9)
Total	684	1344

Odds ratio = 1.18 (0.79 to 1.75), p = 0.397
There was no significant difference between adult cases and controls in the use of antibiotics.

Appendix Table 19: Use of antacids and admission to hospital

Use of antacids	Admission to hospital	
	No (%)	Yes (%)
No	535 (87.4)	55 (80.9)
Yes	48 (7.8)	8 (11.8)
Missing	29 (4.7)	5 (7.3)
Total	612	68

Chi square = 1.43, p = 0.231
There was no significant association among cases between the prior use of antacids and admission to hospital.

Appendix Table 20: Overnight stay outside the study area by adult/Child

Person	Overnight stay outside study area	Case (%)	Control (%)
Adult	No	437 (63.9)	1056 (78.6)
	Yes	243 (35.5)	287 (21.3)
	Missing	4 (0.6)	1 (0.1)
	Total	684	1344
Child	No	75 (71.4)	202 (82.4)
	Yes	30 (28.6)	43 (17.5)
	Total	105	245

For adults: Odds ratio = 2.05 (1.66 to 2.52), p < 0.001
For children: Odds ratio = 1.88 (1.06 to 3.32), p = 0.020
For both children and adults an overnight stay outside the study area was significantly more common among cases than controls.

Appendix Table 21: Cases and controls reporting having a pet adult/child

Person	Pets at home	Case (%)	Control (%)
Adult	No	338 (49.4)	753 (56.0)
	Yes	342 (50.0)	586 (43.6)
	Missing	4 (0.6)	5 (0.4)
	Total	684	1344
Child	No	48 (45.7)	106 (43.3)
	Yes	57 (54.3)	138 (56.3)
	Missing	0	1 (0.4)
	Total	105	245

For adults: Odds ratio = 1.30 (1.08 to 1.57) p = 0.0054
For children: Odds ratio = 0.91 (0.56 to 1.48) p = 0.695
For children there was no significant difference between cases and controls for having a pet at home.

Appendix Table 22: Types of pets reported by cases and controls
Selected for those cases and controls who report having a pet in the household

Pet		Case (%)	Control (%)
Dog	No	99 (24.8)	215 (29.7)
	Yes	236 (59.1)	359 (49.6)
	Missing	64 (16.0)	150 (20.7)
Cat	No	111 (27.8)	243 (33.6)
	Yes	177 (44.4)	320 (44.2)
	Missing	111 (27.8)	161 (22.2)
Bird	No	200 (50.1)	390 (53.9)
	Yes	27 (6.8)	46 (6.4)
	Missing	172 (43.1)	288 (39.8)
Other pet	No	168 (42.1)	279 (38.5)
	Yes	94 (23.6)	222 (30.7)
	Missing	137 (34.3)	223 (30.8)

Appendix Table 23: Any contact with farm animals adult/child

Person	Contact with farm animals	Case (%)	Control (%)
Adult	No	502 (73.4)	948 (70.5)
	Yes	50 (7.3)	77 (5.7)
	Missing	132 (19.3)	319 (23.7)
	Total	684	1344
Child	No	69 (65.7)	185 (75.5)
	Yes	25 (23.8)	22 (9.0)
	Missing	11 (10.5)	38 (15.5)
	Total	105	245

For adults: odds ratio 1.23 (0.83 to 1.81), p = 0.282.
For children: odds ratio 3.05 (1.54 to 6.04), p = 0.000405

Child cases were significantly more likely to report contact with farm animals and child controls. For adults there was no significant difference.

Appendix Table 24: Contact with farm animals and reporting others in the household to be ill with similar symptoms

Selected for the 536 cases that reported that others lived in the same household and for whom a Yes or No response to farm animals was available.

Number of others in the household also ill	Contact with farm animals	
	No (%)	Yes (%)
0	405 (86.7)	56 (81.2)
1	47 (10.1)	11 (15.9)
2	9 (1.9)	2 (2.9)
3	6 (1.3)	0
Total	467	69

Chi square 1.55, p = 0.21

For those living in the same household as other, there as no significant association between contact with farm animals and reporting others in the same household to be ill with similar symptoms.

Appendix Table 25: Consumption of shellfish eg mussels, cockles at home

	Case (%)	Control (%)
No	610 (77.3)	1281 (80.6)
Yes	43 (5.4)	112 (7.0)
Not sure	10 (1.3)	3 (0.2)
Missing	126 (16.0)	193 (12.1)
Total	789	1589

Odds ratio = 0.81 (0.55 to 1.18), p = 0.246

There was no significant association between *Campylobacter* infection and reporting eating shellfish prepared at home.

Appendix Table 26: Drinking bottled water

Drank bottled water	Case (%)	Control (%)
No	333 (42.2)	772 (48.6)
Yes	403 (51.1)	793 (49.9)
Not Sure	43 (5.4)	12 (0.8)
Missing	10 (1.3)	12 (0.8)
Total	789	1589

Odds ratio = 1.18 (0.98 to 1.41), p = 0.067.

There was no significant association between *Campylobacter* infection and reporting drinking bottled water, for the unadjusted odds ratio.

Appendix Table 27: Drinking still bottled water in the 5 days before onset/completing questionnaire

Selected for those participants who reported drinking bottled water.

	Case (%)	Control (%)
No	10 (2.5)	41 (5.2)
Yes	363 (90.1)	681 (85.9)
Missing	30 (7.4)	71 (9.0)
Total	403	793

Chi-square = 4.98, p = 0.0256.

Among cases and controls who drank bottled water, cases were significantly more likely to report drinking still bottled water than cases.

Appendix Table 28: Drinking sparkling bottled water in the 5 days before onset/completing questionnaire

Selected for those participants who reported drinking bottled water.

	Case (%)	Control (%)
No	96 (23.8)	233 (29.4)
Yes	83 (20.6)	191 (24.1)
Missing	224 (55.6)	369 (46.5)
Total	403	793

Chi-square = 0.09, p = 0.766.

Among cases and controls who drank bottled water, there was no significant difference in the drinking of sparkling water.

Appendix Table 29: Drinking water from a drinks dispenser

	Case (%)	Control (%)
No	577 (73.1)	1159 (72.9)
Yes	164 (20.8)	382 (24.0)
Not sure	22 (2.8)	16 (1.0)
Missing	26 (3.3)	32 (2.0)
Total	789	1589

Odds ratio 0.86 (0.70 to 1.07), p = 0.164

There was no significant association between *Campylobacter* infection and drinking water from a drinks dispenser.

Appendix Table 30: Drinking water from a drinking fountain

	Case (%)	Control (%)
No	652 (82.6)	1327 (83.5)
Yes	49 (6.2)	124 (7.8)
Not sure	17 (2.2)	17 (1.1)
Missing	71 (9.0)	121 (7.6)
Total	789	1589

Odds ratio = 0.80 (0.56 to 1.15), p = 0.214

There was no significant association between *Campylobacter* infection and drinking water from a drinking fountain.

Appendix Table 31: Drinking water from a river/stream

	Case (%)	Control (%)
No	697 (88.3)	1426 (89.7)
Yes	8 (1.0)	19 (1.2)
Not sure	8 (1.0)	5 (0.3)
Missing	76 (9.6)	139 (8.7)
Total	789	1589

Odds ratio = 0.86 (0.34 to 2.09), p = 0.725.
There was no significant association between *Campylobacter* infection and drinking water from a river/stream.

Appendix Table 32: Water supplies, where the final coded source used for analysis, differs from that given on the questionnaire

Study ID	Water ID	Source on Questionnaire	Pay council for water	Source from testing team	Justification for change	Final Source assigned
47	35	Not sure	Not Sure	Mains	Mains from water testing, not on EHO list, coded as mains	Mains
204	148	Not sure	Yes	Mains	Mains from water testing, not on EHO list, coded as mains	Mains
300	232	Not sure	Yes	Mains	Mains from water testing, not on EHO city list, coded as mains	Mains
371	292	PWS	Yes	Mains	Mains from water testing, not on EHO list, coded as mains	Mains
400		PWS	Not sure	Not tested	Not on City EHO list, changed to mains	Mains
450		Not sure	Yes	Not tested	Pays council, not on City EHO list, changed to mains	Mains
451		Not sure	No	Not tested	Not on City EHO list, changed to mains	Mains
460		Not sure	Not sure	Not tested	Not on City EO list, changed to mains	Mains
614		Not sure	Yes	Not tested	Pays council for water, not on City EHO list, coded as mains	Mains
640	489	No answer	No answer	Mains	Mains from water testing, not on EHO list, coded as mains	Mains
723		Both	Yes	Not tested	Pays council, not on EHO city list, coded as mains	Mains
811	627	Not sure	Not sure	Mains	Mains from water testing, not on City EHO list, coded as mains	Mains
865		Not sure	Not sure	Not tested	Not on city EHO list, coded as mains	Mains
873	671	Not sure	Not sure	Mains	Mains from water testing, not on City EHO list, coded as mains	Mains
957	720	Not sure	Not sure	Mains	Mains from water testing, not on City EHO list, coded as mains	Mains
1068		Both	Yes	Not tested	Not on city EHO listed, pays council, coded as mains	Mains
1249		Not sure	Not sure	Not tested	Not on city EHO list, coded as mains	Mains
1298	973	Not sure	Not sure	Mains	Mains from water testing, not on City EHO list, coded as mains	Mains
1314	986	No answer	No answer	Mains	Mains from water testing, not on EHO list, coded as mains	Mains
1322		Not sure	Yes	Not tested	Pays council, not on EHO list, coded as mains	Mains
1461		Not sure	Not sure	Not tested	Not on city EHO list, coded as mains	Mains
1467		Not sure	Not sure	Not tested	Not on city EHO list, coded as mains	Mains
1502		No answer	No answer	Not tested	Not on EHO list, coded as mains	Mains
1515		No answer	Yes	Not tested	Pays council, not on EHO list, coded as mains	Mains
1532		Not sure	Yes	Not tested	Pays council, not on EHO list, coded as mains	Mains
1565		Not sure	Not sure	Not tested	Not on EHO list, coded as mains	Mains
1637		Not sure	Yes	Not tested	Pays council, not on city EHO list, coded as mains	Mains

1625		Both	Yes	Not tested	Pays council, not on city EHO list, coded as mains	Mains
1758		No answer	No answer	Not tested	Not on city EHO list, coded as mains	Mains
1792		Private water supply	Not sure	Not tested	Not on city EHO list, not sure about paying council, recoded as mains	Mains
1793		Not sure	Yes	Not tested	Not on city EHO list, pays council, coded as main	Mains
1833		Not sure	Yes	Not tested	Pays council, not on city EHO list, coded as mains	Mains
1857	1004	Both	Not sure	Mains	Tested as mains, not on EHO city list, recoded as mains	Mains
1894		Not sure	Yes	Not tested	Pays council, not on EHO list, coded as mains	Mains
1918		No answer	No answer	Not tested	Not on EHO list, coded as mains	Mains
1971		No answer	No answer	Not tested	Not on EHO list, coded as mains	Mains
1990		No answer	No answer	Not tested	Not on EHO list, address is a farm, coded as mains	Mains
2055		Both	Yes	Not tested	Pays council, not on EHO list not is similar postcode/area, recoded as mains	Mains
2093		No answer	No answer	Not tested	Not on EHO list, coded as mains	Mains
2257		No answer	No answer	No tested	Not on EO list, coded as mains	Mains
2276		Not sure	Yes	Not tested	Pays council, not on EHO list, coded as mains	Mains
2285		Not sure	Yes	No tested	Pays council, not on city EHO list, coded as mains	Mains
2378		Not sure	No	Not tested	Not on city EHO list, coded as mains	Mains
2389		No answer	No answer	Not tested	Not on city EHO list, coded as mains	Mains
2411		No answer	No answer	Not tested	Not on EHO list, coded as mains	Mains
2450		Both	Yes	Not tested	Not on city EHO list, recoded as mains	Mains
2465		Both	Yes	Not tested	Pays council, not on city EHO list, recoded as mains	Mains
2476		Both	Not sure	Not tested	Not on EHO list, recoded as mains	Mains
2503		Not sure	Yes	Not tested	Pays council,	Mains
2521		Not sure	Not sure	Not tested	Not on city EHO, recoded as mains	Mains
2550		Not sure	Yes	Not tested	Pays council, not on EHO city list, coded as mains	Mains
80		No answer	No answer	Not tested	Farm but not croft is on EHO list. No other infor available, code as Not known	Not known
1922	1007	Both	Yes	Mains	Not on EHO list nor is postcode. Tested as mains, coded as mains	Mains
1932		Not sure	No answer	Not tested	House name is on EHO list, coded as PWS. Same house as 1942	Private water supply
1942		Not sure	Not sure	Not tested	House name is on EHO list, coded as PWS. Same house as 1932	Private water supply
2213		Not sure	No	No tested	House name and postcode on EHO list, recoded as PWS	Private water supply
2627	49	Mains	Not sure	PWS	Address on EHO list, sampled as PWS, changed to PWS	Private water supply

Appendix Table 33: Comparison of water source from the questionnaire and the final water source assigned to each participant.

From questionnaire	Final water source used in analysis				Total
	Both	Mains	Not known	Private	
No answer	0	10	1	0	11
Both	4	7	0	0	11
Mains	0	2222	0	1	2223
Not sure	0	22	1	3	26
Private	0	3	0	104	107
Total	4	2264	2	108	2378

No answer to the question of water source was provided on 11 questionnaires, 10 of these could be assigned as mains, one remained unknown.

26 participants reported being not sure of their water source, 22 of these could be assigned as mains, three as private water supplies and one remained unknown.

Eleven participants reported both a mains and private water supply on the questionnaire, after the validation of the database, seven of these were assigned as mains only, with four remaining as both.

For three participants who reported a private water supply on the questionnaire, after data validation this was changed to a mains supply. The remaining 104 reported as private water supply remained as private water supply after the data validation.

Appendix Table 34: Adults on Mains or Private Water supplies

Water supply	Case	Control
Mains	634	1301
PWS	48	40

Odds ratio = 2.46 (1.57 to 3.87) $p < 0.001$
For adults private water supplies were association with a significant risk of *Campylobacter* infection.

Appendix Table 35: Children on Mains or Private Water supplies

Water supply	Case	Control
Mains	89	240
PWS	15	5

Odds ratio = 8.09 (2.65 to 26.32) $p < 0.001$
For children private water supplies were associated with a significant risk of *Campylobacter* infection.

Appendix Table 36: Mains and Private Water Supplies and the age band of cases and controls.

	Age band	Final water source	
		Mains	PWS
Case	<1	5	1
	1-4	35	11
	5-9	29	1
	10-14	15	2
	15-19	34	3
	20-24	57	5
	25-29	43	3
	30-34	40	6
	35-39	44	3
	40-44	74	9
	45-49	55	3
	50-54	53	2
	55-59	83	6
	60-64	59	1
	65-69	36	3
	70-74	24	2
	75-79	20	1
	80+	17	1
Control	<1	9	0
	1-4	84	1
	5-9	63	2
	10-14	61	1
	15-19	75	3
	20-24	78	0
	25-29	74	3
	30-34	89	2
	35-39	109	4
	40-44	151	6
	45-49	131	4
	50-54	136	4
	55-59	154	7
	60-64	118	4
	65-69	93	3
	70-74	62	0
	75-79	35	0
	80+	19	1

Appendix Table 37: Mains and Private Water Supplies and the month of the study.

(Study month defined as the month the questionnaire was received at HPS, for all participants in the pilot and main study).

		Final water source	
		Mains supply	**Private supply**
Case	January	38	3
	February	26	2
	March	27	4
	April	25	0
	May	47	2
	June	75	11
	July	78	7
	August	97	9
	September	87	7
	October	100	6
	November	82	7
	December	41	5
Controls	January	103	2
	February	99	0
	March	107	2
	April	87	1
	May	164	7
	June	127	5
	July	139	3
	August	180	8
	September	171	7
	October	142	3
	November	103	2
	December	119	5

When considering the effect of season on private water supply, there was no significant season by private water supply interaction, (X^2 = 0.097, df = 3, p = 0.992).

Appendix Table 38: Mains and Private Water Supplies by Local authority area.

	Local authority	**Final water source**	
		Mains	**PWS**
Case	Aberdeen City	331	1
	Aberdeenshire	392	62
Control	Aberdeen City	794	0
	Aberdeenshire	747	45

For Aberdeenshire only: Odds ratio 2.63 (1.72 to 4.01), p < 0.001.

Appendix Table 39: Mains and Private Water Supplies and an overnight stay outside the study area in the 14 days before onset (cases) or completing the questionnaire (controls)

	Overnight stay	Final water source	
		Mains (%)	PWS (%)
Case	Not recorded	3 (0.4)	1 (1.6)
	No	461 (63.8)	49 (77.8)
	Yes	259 (35.8)	13 (20.6)
Control	Not recorded	1 (0.1)	0
	No	1222 (80.7)	34 (75.6)
	Yes	318 (21.0)	11 (24.4)

Among those with no history of travel outside the study area the unadjusted odds ratio was 3.82 (2.38 to 6.14), $p < 0.001$, while among those with a history of travel outside the study area, the unadjusted odds ratio was 1.45 (0.60 to 3.54), $p = 0.371$. Therefore having a private water supply was not considered a risk factor among cases who had an overnight stay outside the study area, but was a risk factor for those with no history of an overnight stay outside the study area

Appendix Table 40: Mains and Private Water Supplies and the water having an unpleasant taste

	Unpleasant taste	Final water source	
		Mains (%)	PWS (%)
Case	Not recorded	50 (6.9)	4 (6.3)
	No	652 (90.2)	56 (88.9)
	Yes	21 (2.9)	3 (4.8)
	Total	723	63
Control	Not recorded	35 (2.3)	1 (2.2)
	No	1468 (95.3)	43 (95.6)
	Yes	38 (2.5)	1 (2.2)
	Total	1541	45

Appendix Table 41: Mains and Private Water Supplies and the water having an unpleasant smell

	Unpleasant smell	Final water source	
		Mains	PWS
Case	Not recorded	56 (7.7)	4 (6.3)
	No	657 (90.9)	56 (88.9)
	Yes	10 (1.4)	3 (4.8)
	Total	723	63
Control	Not recorded	49 (3.2)	2 (4.4)
	No	1471 (95.5)	43 (95.6)
	Yes	21 (1.4)	0
	Total	1541	45

Appendix Table 42: Mains and Private Water Supplies and the water being dirty

	Water was dirty	Final water source	
		Mains	PWS
Case	Not recorded	56 (7.7)	2 (3.2)
	No	650 (89.9)	55 (87.3)
	Yes	17 (2.4)	6 (9.5)
	Total	723	63
Control	Not recorded	44 (2.9)	2 (4.4)
	No	1477 (95.8)	42 (93.3)
	Yes	20 (1.3)	1 (2.2)
	Total	1541	45

Appendix Table 43: Is the private water supply chlorinated?
(Selected for participant where the final water source was a private water supply)

Chlorinated	Case (%)	Control (%)
No	7 (11.1)	2 (4.4)
Yes	2 (3.2)	3 (6.7)
Not Sure	0	1 (2.2)
Not recorded	54 (85.7)	39 (86.7)
Total	63	45

Appendix Table 44: Does the private water supply have a UV filter
(Selected for participant where the final water source was a private water supply)

UV filter	Case (%)	Control (%)
No	0	0
Yes	12 (19.0)	12 (26.7)
Not Sure	1 (1.6)	0
Not recorded	50 (79.4)	33 (73.3)
Total	63	45

Appendix Table 45: Other treatment to the private water supply
(Selected for participant where the final water source was a private water supply)

Other treatment	Case	Control
Not recorded	58	41
Coare particle filter	0	1
Filter with granules	0	1
Limestone filter	1	0
Mineral Filter	1	0
Neutraliser	0	1
PH filter	1	1
Rope filter	1	0
RO filter	1	0

Appendix Table 46: Tap water on its own at home, by mains or PWS water source

Water source	Number of glasses	Case (%)	Control (%)
Mains	No	220 (30.4)	395 (25.6)
	Not sure	10 (1.4)	9 (0.6)
	1-3	291 (40.2)	823 (53.4)
	4-6	71 (9.8)	156 (10.1)
	7+	26 (3.6)	45 (2.9)
	Missing	105 (14.5)	113 (7.3)
	Total	723	1541
PWS	No	18 (28.6)	14 (31)
	Not sure	3 (4.8)	0
	1-3	29 (46.0)	22 (48.9)
	4-6	6 (9.5)	4 (8.9)
	7+	4 (6.3)	0
	Missing	3 (4.7)	5 (11.1)
	Total	63	45

Appendix Table 47: Tap water in juice or squash at home

Number of glasses	Case (%)	Control (%)
No	279 (35.4)	555 (34.9)
Not sure	7 (0.9)	3 (0.2)
1-3	241 (30.5)	616 (38.8)
4-6	75 (9.5)	131 (8.2)
7 +	16 (2.0)	41 (2.6)
Missing	171 (21.7)	243 (15.3)
Total	789	1589

Appendix Table 48: Tap water in juice or squash at home, by mains or PWS water source

Water source	Number of glasses	Case (%)	Control (%)
Mains	No	255 (35.3)	536 (34.7)
	Not sure	7 (1.0)	3 (0.2)
	1-3	217 (30.0)	601 (39.0)
	4-6	67 (9.3)	128 (8.3)
	7+	15 (2.1)	40 (2.6)
	Missing	162 (22.4)	233 (15.1)
	Total	723	1541
PWS	No	23 (36.5)	18 (40.0)
	Not sure	0	0
	1-3	23 (36.5)	14 (31.1)
	4-6	8 (12.7)	2 (4.4)
	7+	1 (1.6)	1 (2.2)
	Missing	8 (12.7)	10 (22.2)
	Total	63	45

Appendix Table 59: Tap water on its own at work/school

Number of glasses	Case (%)	Control (%)
No	387 (49.0)	775 (48.8)
Not sure	13 (1.6)	27 (1.7)
1-3	88 (11.1)	216 (13.6)
4-6	16 (2.0)	42 (2.6)
7 +	10 (1.3)	13 (0.8)
Missing	275 (34.8)	516 (32.5)
Total	789	1589

Appendix Table 50: Tap water in juice or squash at work/school

Number of glasses	Case (%)	Control (%)
No	416 (52.7)	874 (55.0)
Not sure	13 (1.6)	18 (1.1)
1-3	36 (4.6)	91 (5.7)
4-6	7 (0.9)	17 (1.1)
7 +	3 (0.4)	4 (0.3)
Missing	314 (39.8)	585 (36.8)
Total	789	1589

Appendix Table 51: Tap water on its own elsewhere

Number of glasses	Case (%)	Control (%)
No	400 (50.7)	885 (55.7)
Not sure	36 (4.6)	19 (1.2)
1-3	96 (12.2)	217 (13.7)
4-6	18 (2.3)	27 (1.7)
7 +	7 (0.9)	4 (0.3)
Missing	232 (29.4)	437 (27.5)
Total	789	1589

Appendix Table 52: Tap water in juice or squash elsewhere

Number of glasses	Case (%)	Control (%)
No	436 (55.3)	913 (57.5)
Not sure	33 (4.2)	13 (0.8)
1-3	54 (6.8)	158 (9.9)
4-6	7 (0.9)	18 (1.1)
7 +	1 (0.1)	6 (0.4)
Missing	258 (32.7)	481 (30.3)
Total	789	1589

Appendix Table 53: Consent to participate in the water testing component by pilot or main study

Consenting to water testing	Main study (%)	Pilot study (%)
No	794 (31.7)	39 (21.4)
Yes	1712 (68.3)	143 (78.6)
Total	2506	182

Appendix Table 54: Consenting to participate in the water testing component by residence in Aberdeen City or Aberdeenshire

Consenting to water testing	Aberdeen City (%)	Aberdeenshire (%)
No	390 (30.2)	443 (31.7)
Yes	900 (69.8)	955 (68.3)
Total	1290	1398

There was no significant difference in consenting to participate in the water testing for those resident in Aberdeen City or Aberdeenshire

Appendix Table 55: Consenting to participate in the water testing component by deprivation category

Consenting to water testing	Deprivation category					
	1 (%)	2 (%)	3 (%)	4 (%)	5 (%)	6 (%)
No	169 (26.0)	266 (32.1)	176 (35.1)	144 (31.0)	28 (28.0)	41 (31.1)
Yes	481 (74.0)	562 (67.9)	325 (64.9)	320 (69.0)	72 (72.0)	91 (68.9)
Total	650	828	501	464	100	132

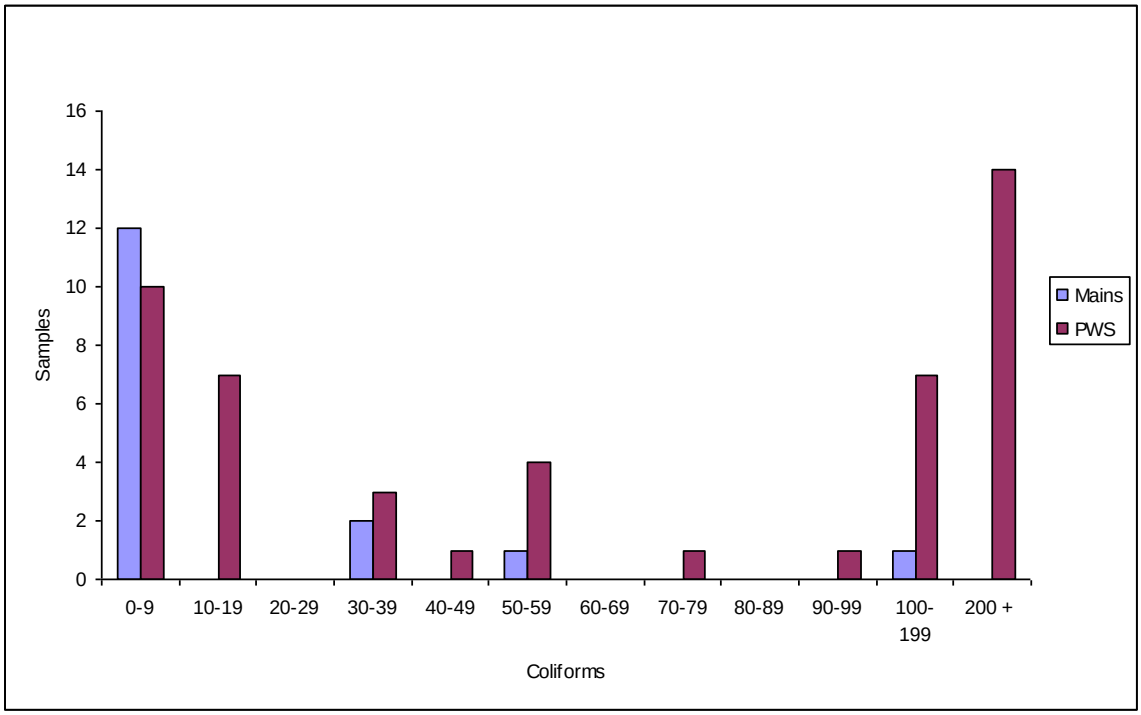
There was no significant difference in consenting to participate in the water testing by deprivation category.

Appendix Table 56: Water testing conducted by type of water supply

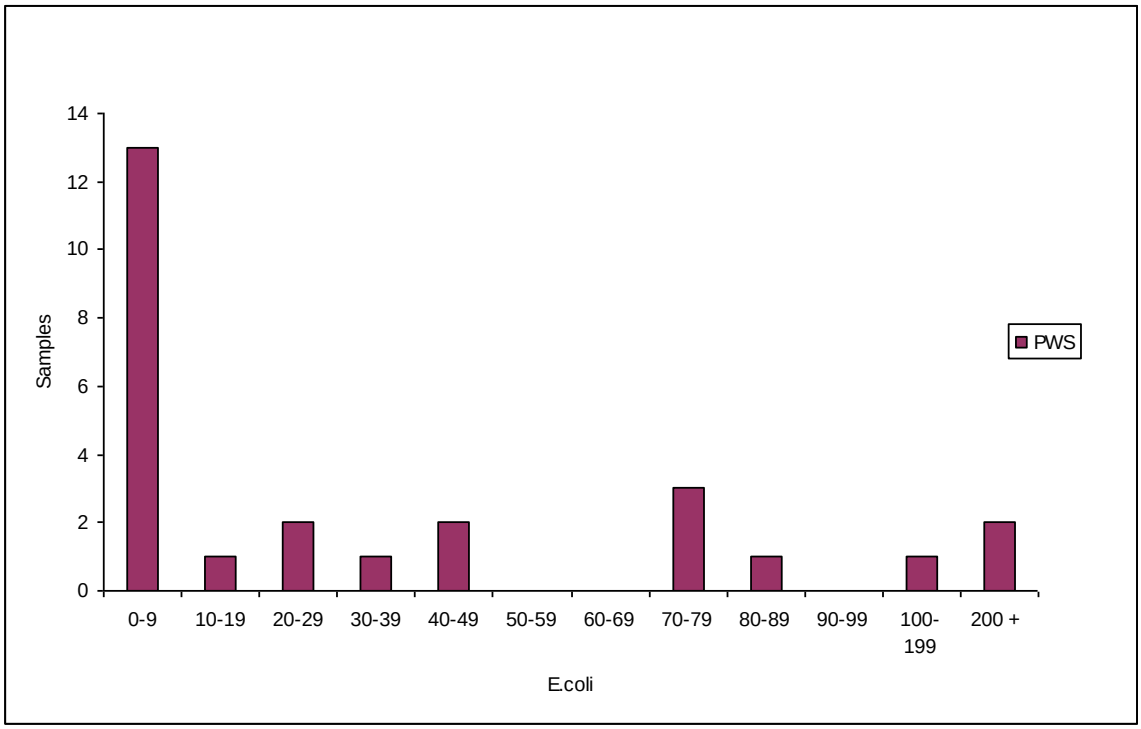
Water testing	Mains (%)	PWS (%)	Both (%)	Not known (%)
Yes	925 (36.2)	77 (62.6)	4 (80.0)	0
No	1633 (63.8)	46 (37.4)	1 (20.0)	2 (100)
Total	2558	123	5	2

(In year 2 of the study, protocol changed to only invite those on a private water supply to participate in the water testing component).

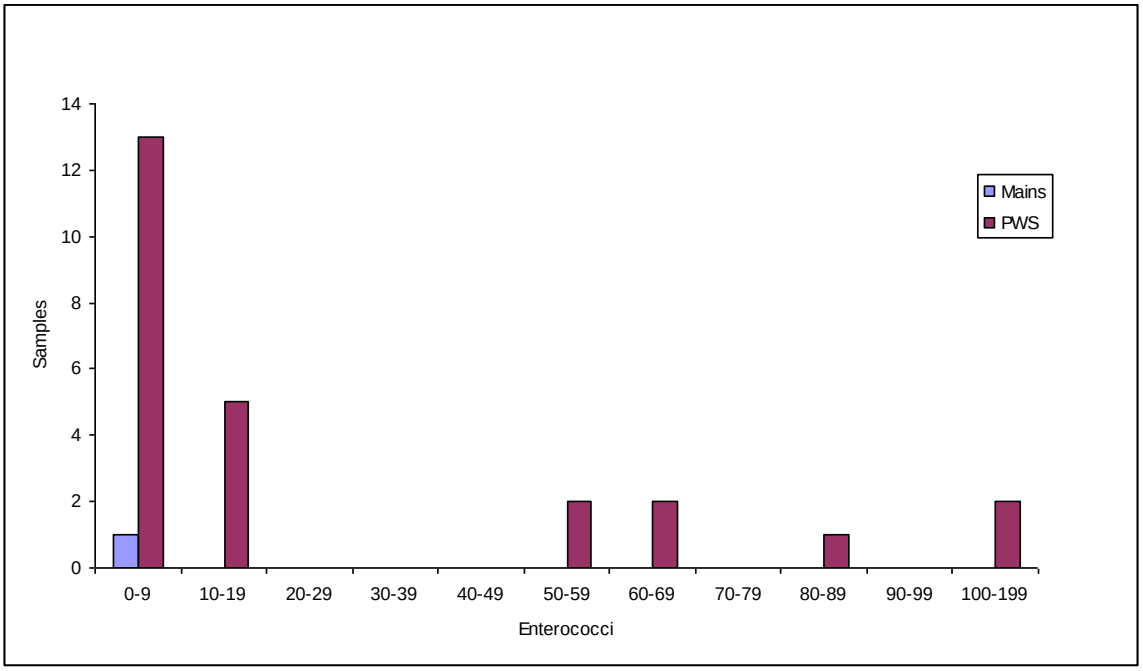
Appendix Figure 1: Number of coliforms from positive mains and private water supply samples



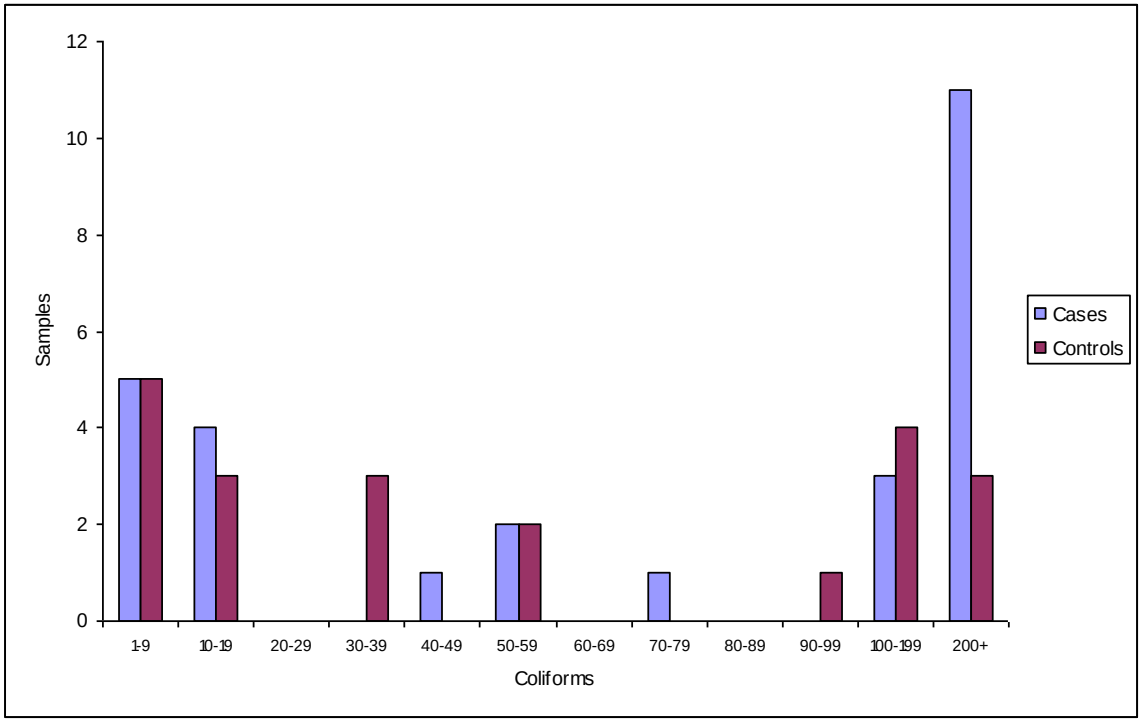
Appendix Figure 2: Number of *E. coli* from positive private water supply samples



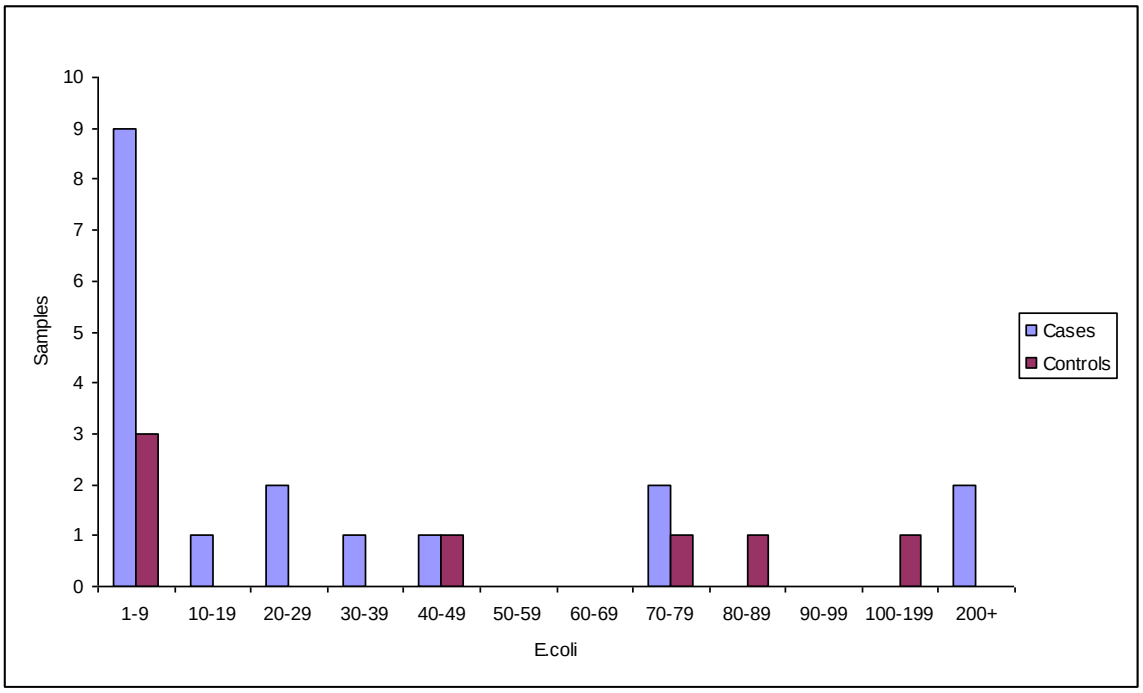
Appendix Figure 3: Number of Enterococci from positive mains and private water supply samples



Appendix Figure 4: Number of coliforms detected in private water supplies from cases and controls



Appendix Figure 5: Number of *E. coli* detected in private water supplies from cases and controls.



Appendix Table 57: Relationship between the detection of coliforms and *E. coli* from private water supplies

Coliforms	***E. coli***	
	No	**Yes**
No	29	0
Yes	23	25

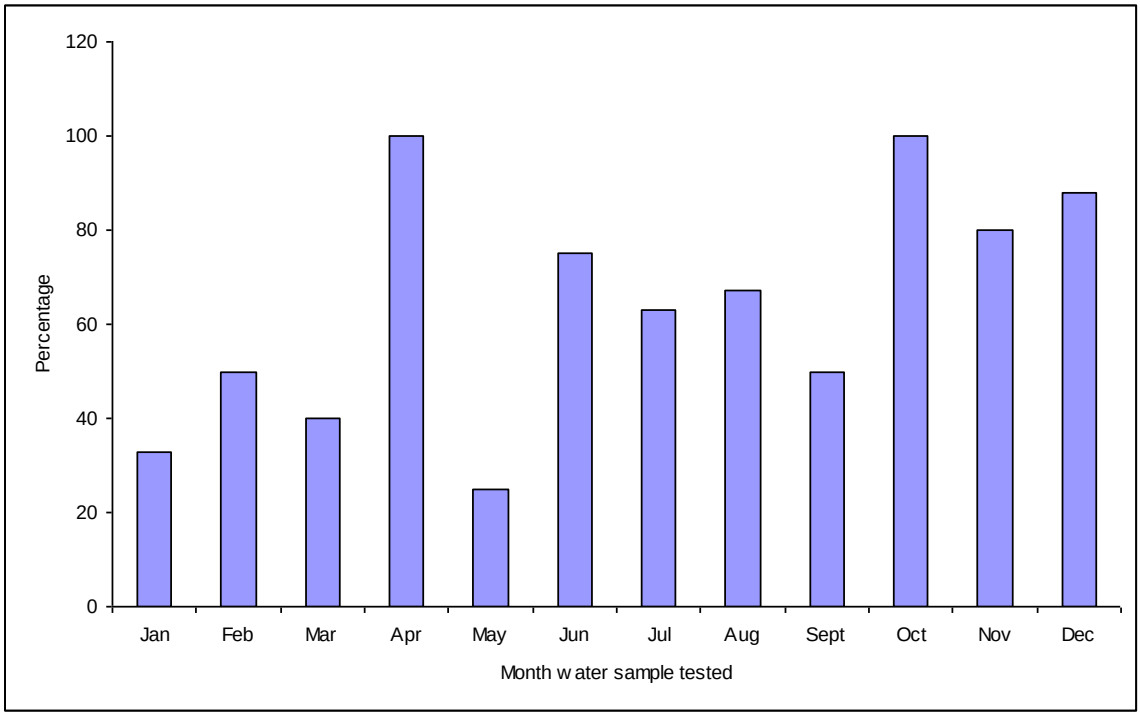
Chi-square = 22.36, df = 1, $p < 0.001$.
There was a significant association between the detection of coliforms and *E. coli* from a private water supply.

Appendix Table 58: Relationship between the detection of coliforms and Enterococci from private water supplies

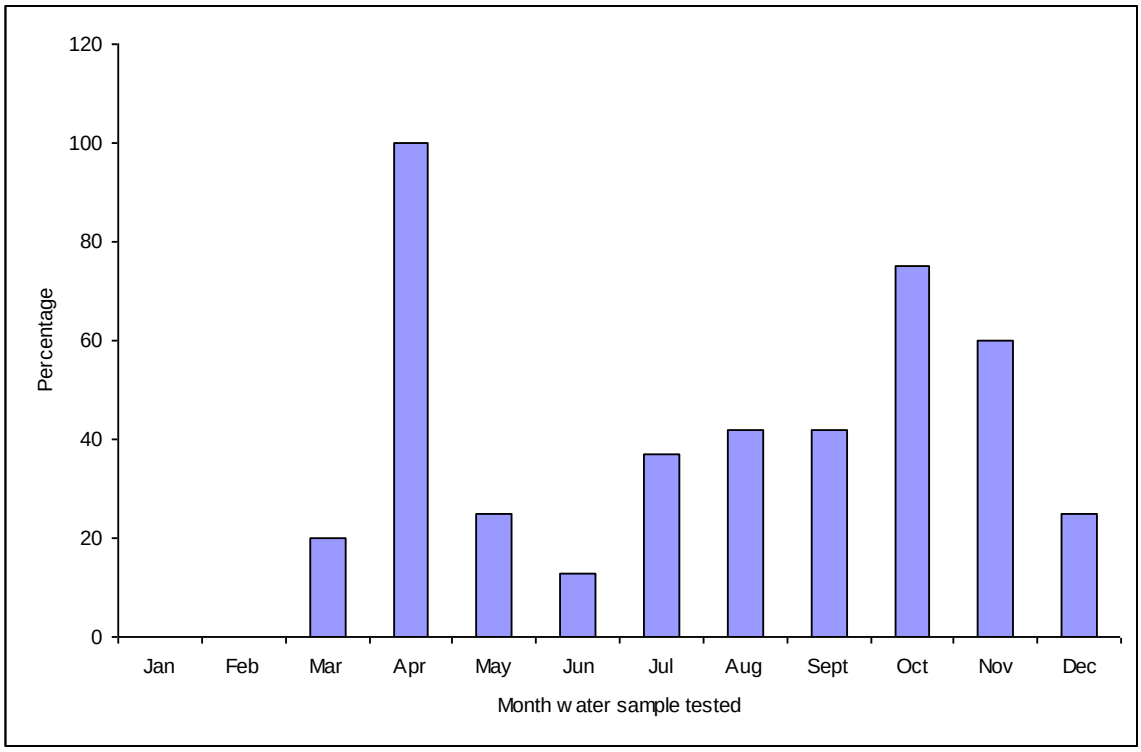
Coliforms	**Enterococci**	
	No	**Yes**
No	27	2
Yes	25	23

Chi square = 13.873, df = 1, $p < 0.001$)
There was a significant association between the detection of coliforms and Enterococci from a private water supply.

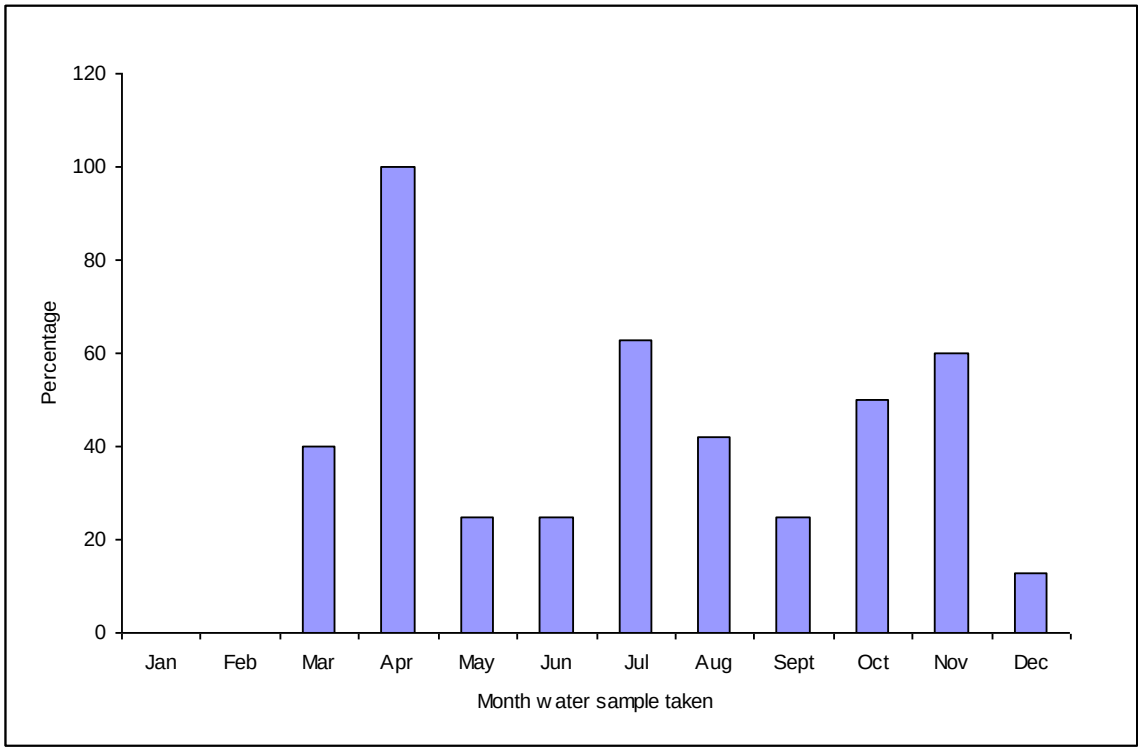
Appendix Figure 6: Percentage of private water supplies in which coliforms were detected by month sample taken



Appendix Figure 7: Percentage of private water supplies in which *E. coli* were detected by month sample taken



Appendix Figure 8: Percentage of private water supplies in which Enterococci were detected by month water sample tested



MLST analysis

Due to the large number of clonal complexes, for the first analysis relationship between CC and the categorical variables using the Pearson Chi-square and Likelihood ratio chi-square methods, the analysis was conducted on CCs ST-206, ST 21, ST 2257, ST 45, ST 48, ST 828 and the others group together.

Appendix Table 59: CC used in analysis against categorical variables

CC	Count
ST 206	45
ST 21	177
ST 257	69
ST 45	77
ST 48	57
ST 828	40
Others	235

Appendix Table 60: CC and month of onset of illness

		Jan/Feb	Jul/Aug	Mar/Apr	May/June	Nov/Dec	Sep/Oct	Missing
Other	Count	13	50	23	43	33	69	4
	Expected count	17.30	59.70	18.32	49.19	25.78	60.72	
	Contribution to Chi square	1.0686	1.5762	1.1972	0.7778	2.0222	1.1297	
ST-206	Count	4	8	6	6	4	14	4
	Expected count	3.15	10.85	3.33	8.94	4.69	11.04	
	Contribution to Chi square	0.2322	0.7507	2.1399	0.9683	0.1008	0.7938	
ST-21	Count	10	52	8	40	20	40	7
	Expected count	12.73	43.94	13.48	36.20	18.97	44.68	
	Contribution to Chi square	0.5859	1.4803	2.2279	0.3996	0.0557	0.4911	
ST-257	Count	9	23	5	11	4	16	1
	Expected count	5.09	17.57	5.39	14.48	7.59	17.87	
	Contribution to Chi square	2.9982	1.6752	0.0285	0.8358	1.6772	0.1964	
ST-45	Count	5	22	5	27	2	14	2
	Expected count	5.62	19.38	5.95	15.97	8.37	19.71	
	Contribution to Chi square	0.0677	0.3533	0.1508	7.6196	4.8479	1.6560	
ST-48	Count	6	14	2	13	6	15	1
	Expected count	4.19	14.47	4.44	11.92	6.25	14.72	
	Contribution to Chi square	0.7779	0.0154	1.3413	0.0972	0.0100	0.0053	
ST-828	Count	4	7	5	5	7	11	1
	Expected count	2.92	10.08	3.09	8.30	4.35	10.25	
	Contribution to Chi square	0.3988	0.9407	1.1766	1.3146	1.6105	0.0547	
All 681	Count	51	176	54	145	76	179	
	Expected count	51.00	176.00	54.00	145.00	76.00	179.00	

Pearson Chi-square = 47.868, DF = 30, p = 0.020
Likelihood Ratio chi-square = 48.720, DF = 30, p = 0.017

There was a significant association between month of illness and CC. It would appear that for example, there were more cases of ST 45 in May/June than expected and less in Nov/Dec

To reduce the number of groups (necessary for chi-squared analysis, the months were grouped eg Jan/Feb etc

Appendix Table 61: CC and overnight stay outside the study area

CC Group	Cell	Overnight stay outside study area			
		No	Yes	Missing	All
Other	Count	143	91	1	234
	Expected count	153.31	80.69		234.00
	Contribution to Chi square	0.6934	1.3174		
ST-206	Count	23	22	0	45
	Expected count	29.48	15.52		45
	Contribution to Chi square	1.4254	2.7984		
ST 21	Count	114	63	0	177
	Expected count	115.97	61.03		177.00
	Contribution to Chi square	0.0333	0.0633		
ST 257	Count	57	11	1	68
	Expected count	44.55	23.45		68.00
	Contribution to Chi square	1.2579	2.3901		
ST 45	Count	57	18	2	75
	Expected count	49.14	25.86		75.00
	Contribution to Chi square	1.2579	2.3901		
ST 48	Count	35	22	0	57
	Expected count	37.34	19.66		57.00
	Contribution to Chi square	0.1472	0.2797		
ST 828	Count	27	13	0	40
	Expected count	26.21	13.79		40.00
	Contribution to Chi square	0.0240	0.0456		
All	Count	456	240		696
	Expected count	456	240		696.00

Pearson Chi square = 20.473, DF = 6, p = 0.002
Likelihood ratio chi-square = 21.809, DF = 6, p = 0.001

There was a significant association between having an overnight stay outside the study area and CC. More cases of ST-257 had an overnight stay outside the study area than expected

Appendix Table 62: CC and travel abroad

CC Group	Cell	Travel abroad		
		No	**Yes**	**All**
Other	Count	179	56	235
	Expected count	195.39	39.61	235.00
	Contribution to Chi square	1.3742	6.7776	
ST-206	Count	28	17	45
	Expected count	37.41	7.59	45.00
	Contribution to Chi square	2.3788	11.6836	
ST 21	Count	151	26	177
	Expected count	147.16	29.84	177.00
	Contribution to Chi square	0.1001	0.4935	
ST 257	Count	66	3	69
	Expected count	57.37	11.63	69.00
	Contribution to Chi square	1.2986	6.4052	
ST 45	Count	72	5	77
	Expected count	64.02	12.98	77.00
	Contribution to Chi square	0.9947	4.9060	
ST 48	Count	52	5	57
	Expected count	47.39	9.61	57.00
	Contribution to Chi square	0.4482	2.2104	
ST 828	Count	34	6	40
	Expected count	33.26	6.74	40.00
	Contribution to Chi square	0.0166	0.0818	
All	Count	582	118	700
	Expected count	582.00	118.00	700.00

Pearson chi-square = 39.159, DF = 6, $p < 0.001$
Likelihood ratio chi-square = 40.200, DF = 6, $p < 0.001$
There was a significant association between travel abroad and CC.

Appendix Table 63: CC and contact with farm animals in 5 days before onset.

CC Group	Cell	Contact with farm animals			
		No	Yes	Missing	All
Other	Count	181	17	37	198
	Expected count	174.44	23.56		198.00
	Contribution to Chi square	0.2463	1.8242		
ST-206	Count	26	8	11	34
	Expected count	29.96	4.04		34.00
	Contribution to Chi square	0.5222	3.8675		
ST 21	Count	124	26	27	150
	Expected count	132.16	17.84		150.00
	Contribution to Chi square	0.5032	3.7270		
ST 257	Count	52	2	15	54
	Expected count	47.58	6.42		54.00
	Contribution to Chi square	0.4114	3.0468		
ST 45	Count	60	3	14	63
	Expected count	55.51	7.49		63.00
	Contribution to Chi square	0.3640	2.6957		
ST 48	Count	44	4	9	48
	Expected count	42.29	5.71		48.00
	Contribution to Chi square	0.0692	0.5123		
ST 828	Count	24	9	7	33
	Expected count	29.07	3.93		33.00
	Contribution to Chi square	0.8856	6.5583		
All	Count	511	69		580
	Expected count	511.00	69.00		580.00

Pearson chi-square = 25.234, DF = 6, p = < 0.001
Likelihood ratio chi square = 24.388, DF = 6, p < 0.001
(2 cells with expected counts less than 5)
The association between CC type and contact with farm animals was highly significant

Appendix Table 64: CC and eating out.

CC Group	Cell	Contact with farm animals			
		No	**Yes**	**Missing**	**All**
Other	Count	75	150	10	225
	Expected count	86.95	138.05		225.00
	Contribution to Chi square	1.642	1.034		
ST-206	Count	11	13	1	44
	Expected count	17.00	27.00		44.00
	Contribution to Chi square	2.119	1.335		
ST 21	Count	74	99	4	173
	Expected count	66.85	106.15		173.00
	Contribution to Chi square	0.765	0.481		
ST 257	Count	32	34	3	66
	Expected count	25.50	40.50		66.00
	Contribution to Chi square	1.654	1.042		
ST 45	Count	36	40	1	76
	Expected count	29.37	46.63		76.00
	Contribution to Chi square	1.497	0.943		
ST 48	Count	16	40	1	56
	Expected count	21.64	34.36		56.00
	Contribution to Chi square	1.470	0.926		
ST 828	Count	18	20	2	38
	Expected count	14.68	23.32		38.00
	Contribution to Chi square	0.749	0.472		
All	Count	262	416		678
	Expected count	262.00	416.00		678.00

Pearson chi-square = 16.128, DF= 6, p = 0.013
Likelihood Ratio chi-square = 16.338, DF = 6, p = 0.012
There was a significant association between CC type and eating out. (This analysis includes those who will have had an overnight stay outside the study area and those who had travelled abroad).

APPENDIX B: ETHICAL APPROVAL


Ethical approval


Research and Development approva

APPENDIX C: CASE QUESTIONNAIRE AND STUDY INFORMATION LEAFLET


Study case information leaflet


Case questionnaire


Campylobacter leaflet

APPENDIX D: REPORT FOR STUDY S14024, PRIVATE WATER SUPPLIES WATER QUALITY, ABERDEEN UNIVERSITY


Water project final report (S14024)