A Case-Control Study of Sporadic Cryptosporidiosis Conducted in Wales and the North West Region of England

Final Report

Correspondence to:
Paul Hunter
Professor of Health Protection
School of Medicine, Health Policy and Practice
University of East Anglia
Norwich, Norfolk
NR4 7JT

Tel: 01603 591004
Fax: 01603 593752

E-mail: paul.hunter@uea.ac.uk

Contents

Project participants

Principle Investigator:

Paul R Hunter, CDSC North West, Chester

Currently; Professor of Health Protection,
School of Medicine, Health Policy and Practice,
University of East Anglia
Norwich NR4 7TJ
Tel: 01603 591004
Email: paul.hunter@uea.ac.uk

Project team:

Sara Hughes, Qutub Syed & Sarah Woodhouse, CDSC Northwest, Chester.

Rachel Chalmers, PHLS Cryptosporidium Reference Unit, Swansea.

Neville Verlander, PHLS Statistics Unit, London.

Members of the Project Steering Group:

Nick Beeching, University of Liverpool, Liverpool

David Drury, Drinking Water Inspectorate, London

Kenton Morgan, University of Liverpool, Liverpool

Roland Salmon, CDSC Wales, Cardiff

Gordon Nichols, Environmental Surveillance Unit, CDSC, London

Charmian Kerr & Keith Osborn, United Utilities

Mark Reacher, CDSC, London

Summary

A case-control study was conducted in the North West of England and Wales to investigate the aetiology of sporadic Cryptosporidiosis. The study examined the risk factors for sporadic cases of *Cryptosporidium* as a whole, but cases were also allocated with genotype data to enable separate investigations of genotype 1 (human) and 2 (cattle) infections.

427 cases and 427 controls completed a postal questionnaire giving details about their recreational activities, contact with infected people, contact with animals and consumption of food and water in the two weeks prior to becoming ill or receiving the questionnaire. It was possible to allocate genotypes to 191 (45%) of cases of which 115 were genotype 1 and 76 genotype 2. For each dependent variable two models were run. In the first model only positively associated risk factors were included (pos model) and for the second model both positively and negatively associated risk factors (pos-neg model) were included.

For cases as a whole, the main significant risk factors were broadly similar to those expected in an outbreak investigation. Three variables were strongly associated $p<0.01$) with illness in both final models: travel outside the UK, contact with another person with diarrhoea and touching cattle. In the pos-neg model eating ice cream and eating raw vegetables were both strongly negatively associated with illness. Several other positively associated variables achieved varying degrees of significance in one model only: never washing fruit or vegetables before consumption, having a medical condition affecting immunity were also strongly associated with illness, the number of times swum in a toddler pool, age, toileting contact with a child under 5 and number of glasses of unboiled tap water drank at home. Eating tomatoes were negatively associated with illness at the $p< 0.05$ level.

For genotype 1 infections, the strongly significant risk factors were travel abroad, and changing nappies of children under 5, though contact with an infected person was also significant in the positive only model. For genotype 2 infections, the only strongly significant risk factor was contact with farm animals, though eating raw vegetables and tomatoes were both strongly negatively associated with risk of illness.

Conclusions of the study note that the epidemiology of type 1 and 2 infections appear to be different. Epidemiological studies that combine the two pathogens therefore risk being misleading.

Although the number of glasses of mains drinking water drunk each day achieved significance in one model, no other marker of water consumption did in any model and so these results do not support the suggestion that consumption of mains drinking water as an important risk factor for sporadic cryptosporidiosis. It is possible for a variable to be significant in a model purely by chance.

Introduction

Cryptosporidiosis is due to infection by one or more species of the genus *Cryptosporidium*. *Cryptosporidium* is a coccidial protozoan parasite that was first described in the first decade of the last century. About 11 species are now recognised of which *C. parvum* is the most important pathogen for man. It is now recognised that there are two main genotypes of *C. parvum*, type 1 or human type (H) and type 2 or cattle type (C). Genotype 1 is reported as being largely restricted to humans, and genotype 2 is found in a wide range of animals (particularly cattle and sheep) as well as man. There have been many reviews of cryptosporidiosis in the recent past either undertaken by government expert committees (Department of the Environment and Department of Health 1990, Department of the Environment and Department of Health 1995, Department of the Environment, Transport and the Regions, and Department of Health 1998), or others (Meinhardt, Casemore and Miller 1996; Hunter 1997; Kosek et al. 2001; Chen et al. 2002).

Cryptosporidiosis has become the most common protozoal cause of acute gastroenteritis in England and Wales with the number of reports to the Public Health Laboratory Service Communicable Disease Surveillance Centre (PHLS CDSC) being between 4000 and 6000 cases in most years (figure 1). In the North West Region of England there are usually about 1000 cases per annum and in Wales there are usually about 300 cases per annum.

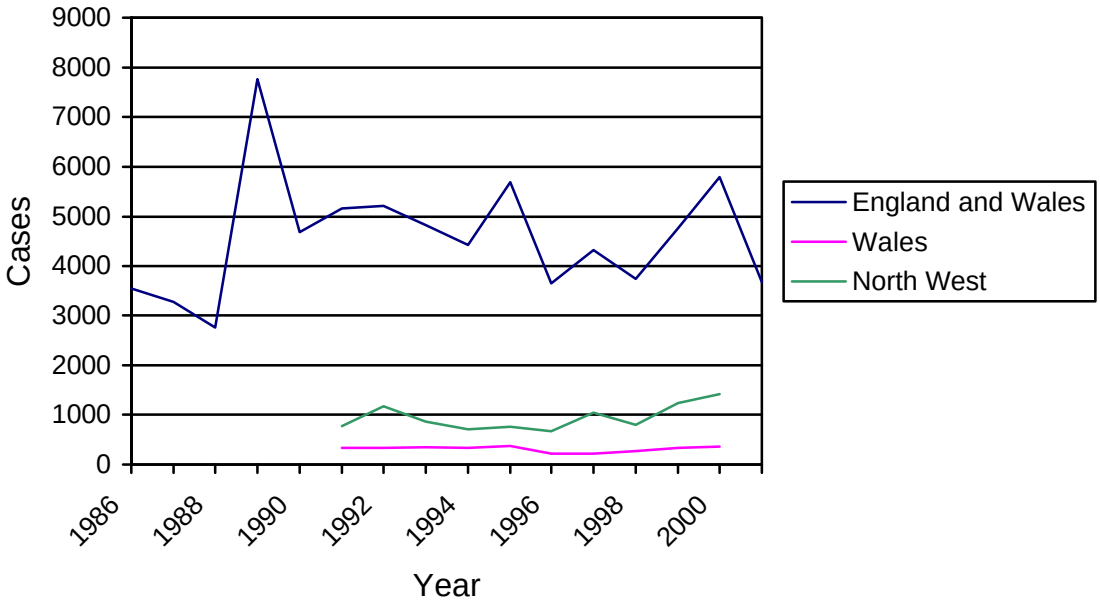


Figure 1. Reported cases of Cryptosporidium in England and Wales by year1986 to 2001. Also showing annual reports from Wales and the North West Region of England (Public Health Laboratory Service Data).

In otherwise healthy individuals, infection with *Cryptosporidium* usually causes a self-limiting diarrhoeal disease. The incubation period is normally about 7 to 10 days (range 4-28 days) and symptoms can last for between 2 and 26 days or occasionally even longer. The main feature is watery diarrhoea that can vary from relatively mild to quite severe. Patients may also complain of abdominal pain and a few also have a mild fever.

In certain immunocompromised individuals, such as those suffering with AIDS, severe combined immunodeficiency syndrome or similar disease that depresses CD4 counts, the disease is usually much more severe and more persistent. (Hunter and Nichols 2002). Illness can last for several months or until death. Severe diarrhoea is associated with marked weight loss. Malaise and fever is also more common. Non-gastrointestinal illness, such as cholecystitis, hepatitis and respiratory disease, may also occur in such individuals.

The risk of infection is greatest in the first five years of life and declines throughout childhood and subsequent adulthood (figure 2)

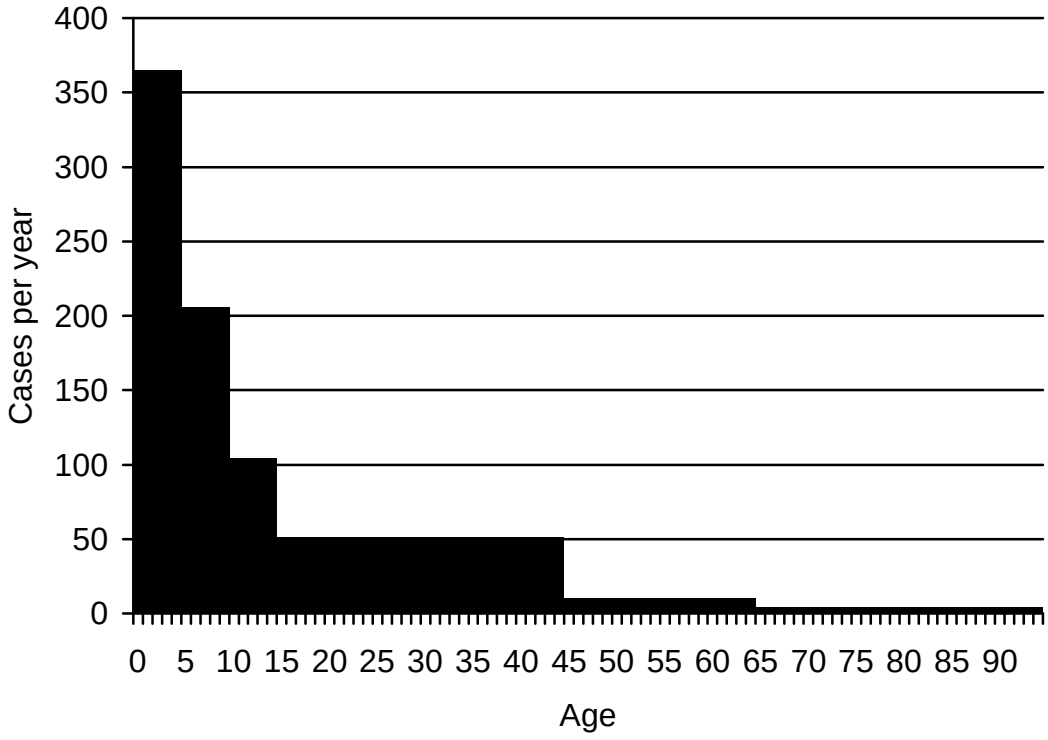


Figure 2. Age distribution of cases of reports of Cryptosporidium infection in England and Wales year 2000 (Public Health Laboratory Service Data).

Individual infections may be associated with outbreaks of disease or occur sporadically. During the years 1992 to 2001 there were 77 outbreaks of cryptosporidiosis reported to CDSC. The identified cause of these outbreaks are listed in table 1. Table 2 shows the proportion of cases associated with outbreaks during those years. Over the time period outbreaks contributed only 7.3% on average of all reported infections, though outbreaks associated with drinking water contributed about 84.4% of these outbreak-related cases (6.1% of all cases).

Table 1. *Cryptosporidium* spp. outbreaks in England and Wales reported to the Gastrointestinal Diseases Division, PHLS Communicable Disease Surveillance Centre, 1992-2001 (n=77). (Unpublished data from CDSC)

Mode of transmission	**1992**	**1993**	**1994**	**1995**	**1996**	**1997**	**1998**	**1999**	**2000**	**2001**	**Total**
Drinking water	5	3	2	1	3	4	2	2	1		23
Direct animal contact	1	2	1	2		2		1	1		10
Person-to-person						1			1	1	3
F'borne followed by person-to-person			2								2
Foodborne				1							1
Recreational water contact											
Swimming pool	1	2	3		1	1	3	7	6	3	27
Beach										1	1
Paddling pool				1							1
River/Stream						1			1		2
Unknown	2	1		1	1	1		1			7
Total	9	8	8	6	5	10	5	11	10	5	77

Table 2 *Cryptosporidium* spp. outbreaks in England and Wales reported to the Gastrointestinal Diseases Division, PHLS Communicable Disease Surveillance Centre, 1992-2001 (n=77) showing total numbers for each year and cases represented as a percentage of all cases. (Unpublished data from CDSC)

Year	Cryptosporidium CoSurv reports*	All Outbreaks	Total +ve All outbreaks	% confirmed All outbreaks**	Drinking water outbreaks	Total +ve drinking water outbreaks	% confirmed drinking water outbreaks**
1992	5166	9	421	8.1	5	343	6.6
1993	4755	8	327	6.9	3	164	3.4
1994	4504	8	375	8.3	2	257	5.7
1995	5703	6	628	11.0	1	575	10.1
1996	3590	5	272	7.6	3	226	6.3
1997	4394	10	811	18.5	4	777	17.7
1998	3673	5	94	2.6	2	62	1.7
1999	5052	11	252	5.0	2	375	7.4
2000	5823	10	152	2.6	1	58	1.0
2001	3630	5	31	0.9	0	0	0.0
Total	46290	77	3363	7.3	23	2837	6.1

* Laboratory confirmed *Cryptosporidium* spp. isolates reported by clinical microbiology laboratories in England and Wales to CDSC.
** Total number of laboratory-confirmed *Cryptosporidium* cases involved in outbreaks as a % of total number of laboratory-confirmed *Cryptosporidium* isolates reported to CDSC in England and Wales.

Most of what we know about the risk factors for *Cryptosporidium* infection comes from the investigation of outbreaks. Outbreaks in the UK have been associated with consumption of drinking water (from public and private supplies), from swimming at swimming pools, consumption of unpasteurised milk, and contact with farm animals (especially during farm visits).

However, the major part of the burden of disease associated with cryptosporidiosis is due to sporadic rather than outbreak-associated infections. Outbreaks represent less than 10% of all cases of *Cryptosporidium* infection (Djuretic et al. 1996; Evans et al. 1998). Although it is likely that a further proportion of cases will be associated with undetected outbreaks (Hunter, Syed and Naumova 2001), one should be cautious about extrapolating from evidence of causation in outbreaks to the causation of sporadic disease. There have been very few studies that have studied sporadic disease specifically. Indeed there is only one substantive case-control study of sporadic cryptosporidiosis conducted in a developed nation reported to-date and that was done in Australia (Robertson et al. 2002).

This report concerns a large case-control study conducted in the North West Region of England and in Wales. The study was designed to investigate the aetiology and epidemiology of sporadic Cryptosporidiosis. The North West Region has a history of a several large waterborne outbreaks of cryptosporidiosis over the past decade, whilst Wales has not had any reported waterborne outbreaks.

The principal hypotheses being tested in this study relate to what we know about the epidemiology of outbreaks, namely that sporadic cases of cryptosporidiosis are associated with:

1. Consumption of unboiled mains drinking water
2. Swimming in a swimming pool
3. Contact with animals
4. Travel outside the UK
5. Contact with other people with infection

Methods

A case-control study was conducted in the North West of England and Wales from February 2001 to May 2002. The study received ethical approval from the Multi-centre Research Ethics Committee (MREC), relevant Local Research Ethics Committees (LRECs) and the PHLS Research Ethics Committee.

Case-control recruitment

Participants were recruited via an enhanced surveillance of *Cryptosporidium* that had commenced in the North West of England and Wales in December 2000. As part of routine procedure, microbiology laboratories sent reports of confirmed cases to the relevant Health Authority. For the enhanced surveillance, details of the confirmed cases were forwarded to CDSC North West, via the Consultant in Communicable Disease Control (CCDC).

The case definition was a laboratory confirmed case of *Cryptosporidium* in a resident of Wales or North West region with diarrhoea in the two weeks before a sample was taken, and which was not part of a formal outbreak investigation. All cases notified to CDSC North West within four weeks of the date of notification to the Health Authority were invited to take part in the study. Notifications exceeding four weeks were excluded as these cases may have had difficulty accurately recalling their activities before becoming ill.

The definition of a control was a person who had not suffered from *Cryptosporidium* in the two weeks before completing a questionnaire. Controls were chosen to be within the same age band as the case and within the same location, being drawn from the same GP or neighbouring GP catchment area. The age bands chosen were: < 5 years old, 5 - 16 years old and > 16 years old. Expecting control participation to be comparatively low, we attempted to recruit eight controls for each participating case.

We recruited controls via the GP of the case, who was identified either by the CCDC upon notification, or from the case's completed questionnaire. We contacted the GP

initially by post. If no response was received, we contacted the practice manager by telephone. We asked consenting GP's to randomly select eight patients of a given age band from their practice list. GP's had the option of 1) writing to the patients and inviting them to take part in the study (a template letter and £ 40 reimbursement for administration costs were provided) or 2) forwarding names and addresses of the patients to CDSC North West. The second option was an addition implemented four months into the study following low GP participation and MREC approval. If a GP did not wish to take part, we contacted a neighbouring practice.

A total of 662 cases and 820 controls were invited to take part in the study. They received a postal questionnaire and an accompanying information leaflet (appendix B), which explained the nature of the study and gave some basic information about cryptosporidiosis. If no response had been received after two weeks, a second questionnaire was sent. After this time it was assumed the person did not want to take part in the study.

The questionnaires were developed for both adult and child cases and controls, where a person below the age of 16 was defined as a child and a person aged 16 or over defined as an adult. The questionnaires were loosely based on that suggested by the Bouchier report for the investigation of sporadic cases, and included information on demographics, occupation, details of illness, contact with people suffering from diarrhoea, travel both within the country and abroad, recreational activities, contact with zoo and farm animals and consumption of food and water. Questionnaires and information leaflets were available primarily in English and Welsh, but additionally in Urdu and Gujarati to include the main ethnic minority communities in Greater Manchester (Copies of the Questionnaires are included in appendix A)

Finally, details of all cases taking part in the study were sent to the PHLS Cryptosporidium Reference Unit in Swansea, where they allocated cases with genotype data.

Genotyping

At the start of the study all laboratories in the North West and in Wales were asked to send positive stools to the PHLS Cryptosporidium Reference Unit in Swansea for typing.

Confirmation

To confirm the identification of Cryptosporidium at the Cryptosporidium Reference Unit, faecal smears were stained using a modified Ziehl-Neelsen stain (Anon, 1998) and inspected by bright field microscopy, or using an auramine phenol method (Casemore, 1991) and inspected by fluorescence microscopy. Equivocal results were confirmed by immunofluorescence antibody test (TCS Water Sciences, Buckingham, UK) according to the manufacturer's instructions.

Genotyping

Prior to DNA extraction, oocysts were purified from the faeces using salt flotation (Ryley et al., 1976). Briefly, the oocysts were separated by flotation from faecal debris using saturated salt solution and centrifugation for 8 minutes at 1600xg. The floated material containing the oocysts was washed with de-ionised oocyst-free water, the oocysts resuspended in 1ml deionised, oocyst-free water and stored at +4^{o}C prior to use. To extract DNA, 200ìl oocyst suspension was incubated at 100ºC for 60 minutes and DNA extracted using proteinase K digestion in lysis buffer at 56ºC and a spin-column filtration technique (QiAMP DNA mini kit, Qiagen, Crawley, UK). DNA extracts were stored at –20^{o}C prior to use.

The Cryptosporidium genotype was investigated using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) to identify polymprophisms within the *Cryptosporidium* Oocyst Wall Protein (COWP) gene locus (Spano et al., 1997). Briefly, primers cry15 and cry9 were used to amplify a 553 base pair region of the COWP gene, which was then subjected to restriction endonuclease digestion by *Rsa*I (Promega, Southampton, UK). The digestion products were separated by agarose (3% w/v) gel electrophoresis and the product size was confirmed by comparison with

a DNA molecular weight standard marker (Life Technologies, Glasgow, UK). The digested products were visualised using ethidium bromide (0.1mg/100ml) and recorded using a digital camera and KDS1D analysis software (Kodak, Rochester, NY, USA). .

All procedures were subject to internal and external quality control, with previously characterised positive and negative control material included in each processing batch.

Data analysis

Data entry was done using Epi-Info. Initial analyses on the clinical severity and presentation were done using SPSS. The statistical modelling of risk factors was done by the PHLS Statistics Unit, using Epi-Info and GLIM.

For the aetiological analyses each potential risk factor was considered singly by its odds ratio estimate (and 95% confidence interval). Continuity corrected chi-square tests or Fisher's exact test was used where the data were sparse. Dose response was estimated using chi-square tests for trends.

Variables that were positively associated with illness (with a p value of 0.2 or less) were included in an initial logistic regression model. The variable representing whether a child ate soil was removed first as this had the most missing data for a non-significant variable and its removal resulted in many more observations available for model estimation. Terms were assessed by comparison of nested models using likelihood ratio tests. Non-significant variables ($p > 0.05$) were removed one at a time from models, with the most insignificant ones being removed first. This resulted in a final multivariable model, with most variables being significant or close to significant. The only case where this did not occur was for genotype 1, where the age variable was retained despite its non-significance.

Of the cases that were genotyped, separate multivariable analyses for type 1 and type 2 were performed using all controls. The set of variables for inclusion into initial multivariable models were determined using all the data, as discussed above.

The analyses were then re-run using all variables, whether positively or negatively associated with illness, with a p value of 0.2 or less. However, it was not possible to add all the variables, as there were too many for the statistical package to handle. Thus all the risk factors and some of the protective factors were included in the initial model. The most insignificant variable was removed and another protective factor included. This process continued until all the protective factors had been included. Then a sequence of models were fitted, on each occasion dropping the most insignificant variable.

Results

Completed questionnaires were received from a total of 427 cases (65% response rate) and 427 controls (52% response rate). Of the controls, 27 (6%) had experienced diarrhoea in the two weeks prior to completion and were excluded from the analysis. Of the cases, 191 (45%) were able to be allocated genotype data; 115 with genotype 1 and 76 with genotype 2.

The median age for recruited cases and controls was 12 years. 48% of cases and 48% of controls were male. The age distribution of cases and controls are shown in figures 3a, 3b, 3c and 3d which give the average age for five or ten year age bands. It is notable that there were some differences in the age distribution between cases and controls, though this was not surprising given that controls were only matched very loosely to broad age bands.

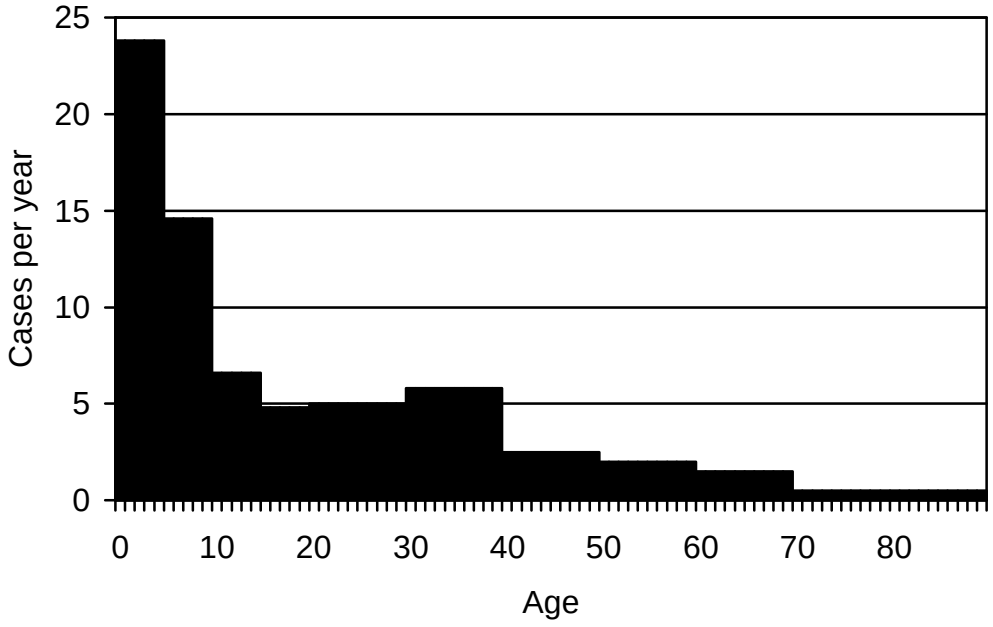


Figure 3a. Age distribution of cases

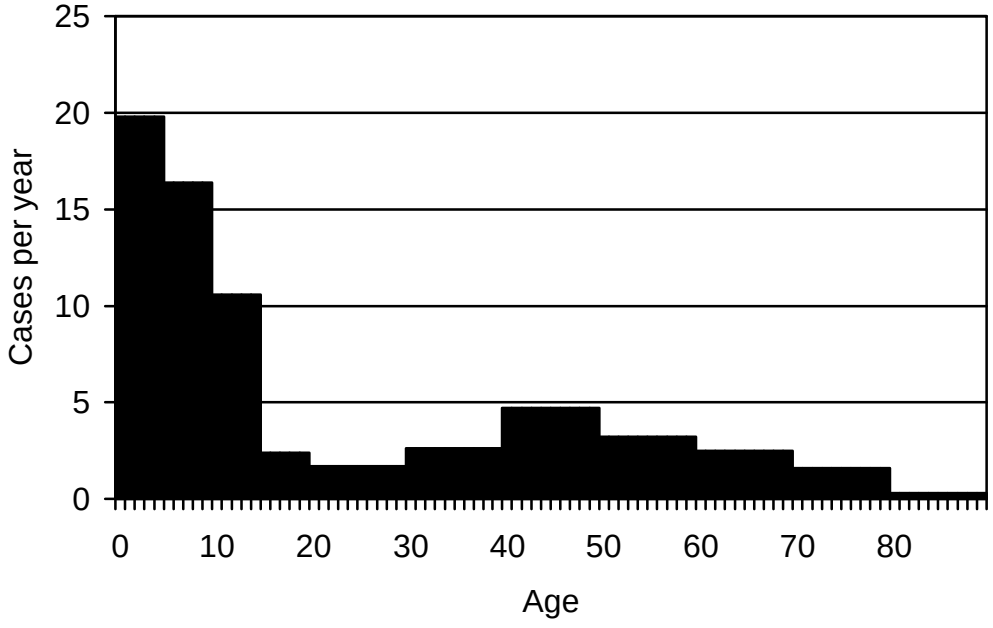


Figure 3b. Age distribution of controls

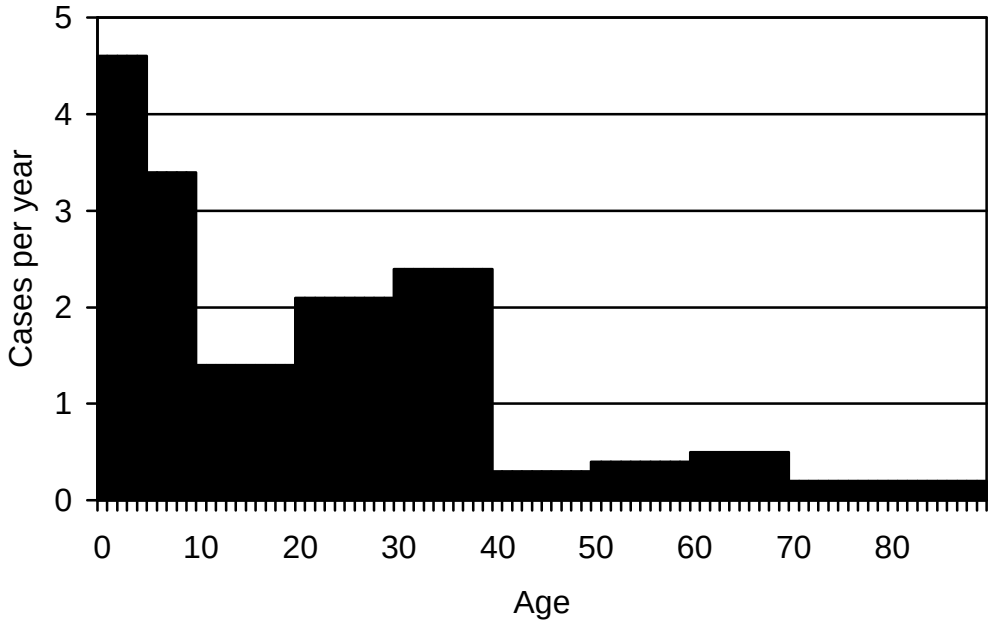


Figure 3c Age distribution of cases with genotype 1 infections

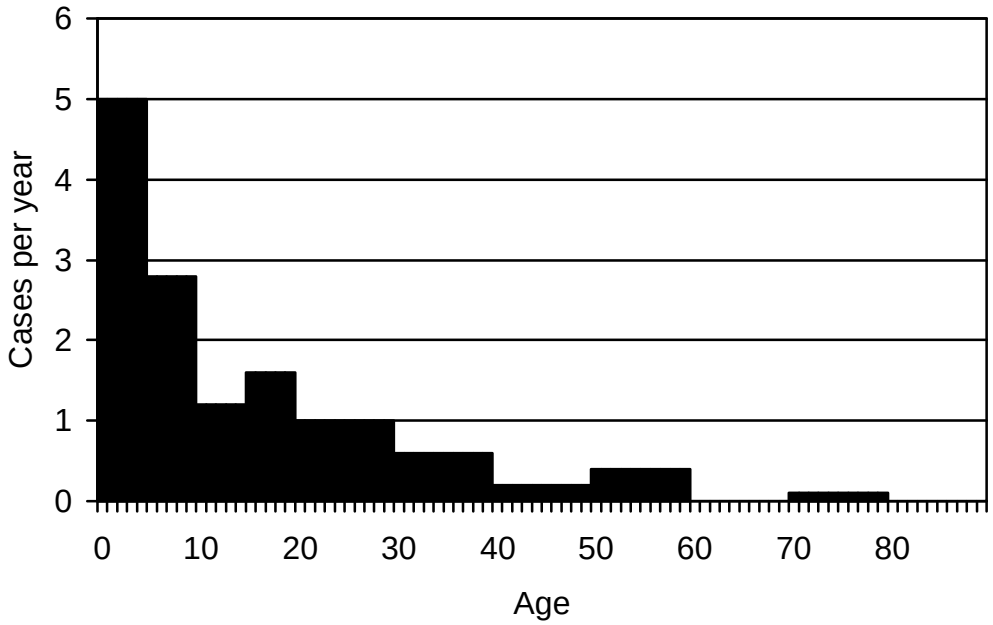


Figure 3d Age distribution of cases with genotype 2 infections

A single variable analysis of age as a continuous variable indicated an association with illness (p=0.007) with decreasing risk of illness with increasing age (estimated odds ratio = 0.991 with 95% CI 0.985 to 0.998). Appendix C shows the single variable analysis results.

Interestingly there was a marked difference in the age distribution between the cases with type 1 and type 2 infections. The median age for people with genotype 1 infection was 21 years and for genotype 2 this was 9 years (p=0.0036, Mann-Whitney U test) (figures 3c and 3d). This was largely due to a second peak of infections in 20s and 30s seen in genotype 1 infections, but not in genotype 2 infection.

Regarding clinical details for cases, 251 (59%) reported fever, 410 (96%) abdominal pain, 279 (65%) vomiting, 49 (11%) bloody diarrhoea and 130 (30%) reported other symptoms. 61 cases (14%) were admitted to hospital with the median number of days stay being 3 (range 1-9). There were no significant differences between genotype 1 or 2 in reported symptoms or whether patients were admitted to hospital.

The duration of illness for total cases (figure 4) showed a mean of 12.7 days. For cases with genotype 1 (figure 5), the mean duration was 13.5 days (SD 9.93). For genotype 2 (figure 6), mean duration was 11.33 days (SD 5.29). Levene's Test for Equality of Variances showed that variance of duration for genotype 2 was significantly lower than genotype 1 (F=8.312, p=0.005). However the difference in mean duration was not significant.

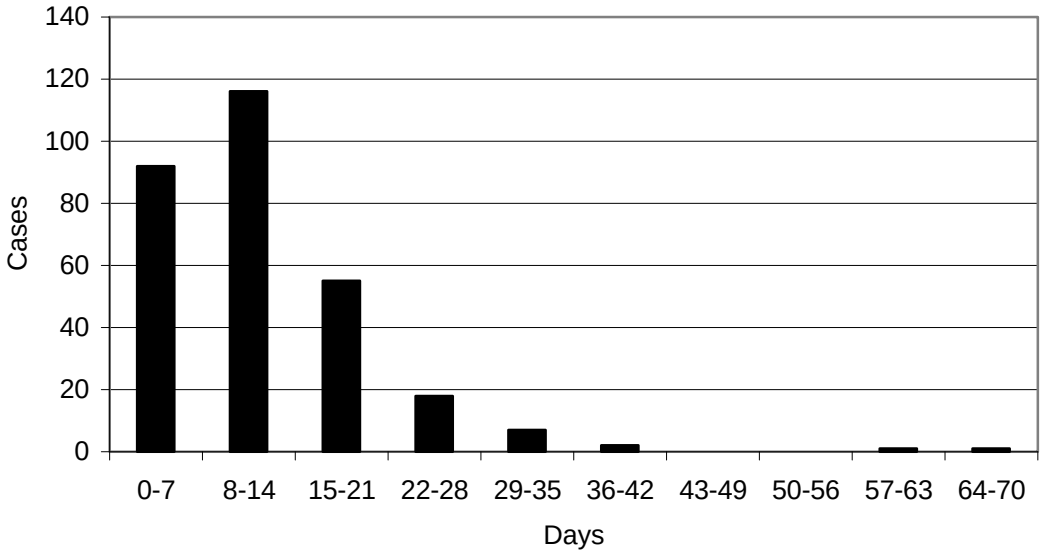


Figure 4. Duration of illness – all cases.

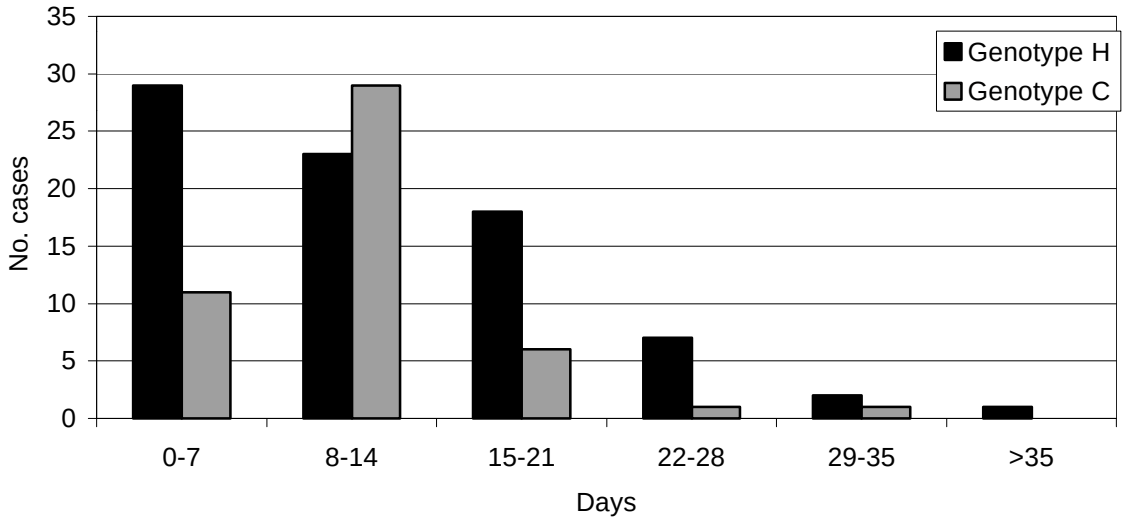


Figure 5. Duration of illness - genotype 1 and genotype 2

Table 3 shows the multivariable results for all data using only positively associated risk factors, estimated from 634 observations. The risk of sporadic cryptosporidiosis appears to vary significantly with health authority, but this is due to just six of the health authorities, four in North West England and two in Wales that appear to have significantly lower risk than Bury and Rochdale. The risk decreases significantly with age.

Not surprisingly, having contact with another person having diarrhoea significantly enhances the risk of being a sporadic case of cryptosporidiosis. Touch any cattle, travelling outside the UK, never washing raw vegetables or fruit prior to eating and frequency of swimming in a toddler pool also appear to be significant risk factors. Having a medical condition known to affect immunity was also a risk factor, though relatively just 4% of cases reported suffering from such a condition. The diagnosis is known for 8 of these cases and none of these diagnoses were of conditions normally thought to be a risk factor for cryptosporidiosis; 2 with Crohn's disease and 1 each with chronic myeloid leukaemia, coeliac disease, cancer, stroke, diabetes and scoliosis.

Table 4 shows the multivariable results for all data using both positively and negatively associated risk factors, estimated from 552 observations. In this model the Health Authority, travel outside the UK, contact with another person with diarrhoea, touch any cattle, were highly significant ($p<0.01$) positive risk factors as in the model described above. 'Toileting contact with a child under 5 years of age' and the number of glasses of unboiled water drunk at home were also significant ($p<0.05$). In addition eating ice cream and raw vegetables were both highly significantly negative associations and eating tomatoes was also significant. Variables significant in the model of only positive risk factors that did not achieve significance in model with both positive and negative variables were age, medical condition affecting immunity, number of times swum in a toddler pool, and never washing raw fruit and vegetables.

Table 3. Final multivariable model (positively associated variables only in initial multivariable model) – all data. Estimated from 634 observations.

		Adjusted Odds Ratio	95% Confidence Interval	p value
Health Authority	Bury and Rochdale	1.000		<0.001
	East Lancashire	0.070	0.023, 0.217	
	Liverpool	n.e.	n.e.	
	Manchester	0.371	0.149, 0.921	
	Morecambe Bay	1.262	0.203, 7.839	
	North West Lancashire	0.146	0.058, 0.369	
	North Cheshire	0.306	0.079, 1.181	
	Salford and Trafford	1.592	0.438, 5.791	
	Sefton	n.e.	n.e.	
	South Cheshire	0.300	0.126, 0.718	
	South Lancashire	158.0	0, ∞	
	St Helens and Knowsley	0.557	0.077, 4.026	
	Stockport	0.543	0.216, 1.364	
	West Pennine	1.074	0.347, 3.320	
	Wigan and Bolton	0.395	0.140, 1.113	
	Wirral	0.681	0.179, 2.589	
	Bro Taf	n.e.	n.e.	
	Dyfed Powys	0.227	0.078, 0.667	
	Gwent	0.125	0.034, 0.465	
	Lechyd Morgannwg	0.300	0.076, 1.187	
	North Wales	0.498	0.212, 1.168	
Age		0.990 per year	0.981, 0.999	0.026
Medical condition affecting immunity	Y	7.501	1.934, 29.09	0.001
	N	1.000		
No. of times swum in a toddler pool		1.252 per time	1.032, 1.520	0.012
Swallow water while in a river	Y	7.068	0.696, 71.79	0.063
	N	1.000		
Travel outside the UK	Y	3.529	1.831, 6.801	<0.001
	N	1.000		
Contact with another person with diarrhoea	Y	3.392	1.929, 5.967	<0.001
	N	1.000		
Touch any cattle	Y	3.673	1.414, 9.543	0.005
	N	1.000		
Touch any farm animals (other than equines, cattle, sheep or fowl)	Y	6.717	0.703, 64.13	0.055
	N	1.000		
Usually wash before eating raw fruit and vegetables	Always	1.000		0.001
	Usually	0.724	0.470, 1.115	
	Sometimes	0.730	0.442, 1.204	
	Never	3.404	1.533, 7.556	

n.e. not estimable

Table 4. Final multivariable model (positively and negatively associated variables in initial multivariable model)– all data. Estimated from 552 observations.

		Adjusted Odds Ratio	95% Confidence Interval	p value
Health Authority	Bury and Rochdale	1.000		0.004
	East Lancashire	0.125	0.041, 0.382	
	Liverpool	n.e.	n.e.	
	Manchester	0.482	0.166, 1.398	
	Morecambe Bay	1.610	0.247, 10.49	
	North West Lancashire	0.225	0.080, 0.635	
	North Cheshire	0.326	0.068, 1.552	
	Salford and Trafford	0.921	0.261, 3.250	
	Sefton	n.e.	n.e.	
	South Cheshire	0.310	0.117, 0.822	
	South Lancashire	316.6	0, ∞	
	St Helens and Knowsley	0.175	0.012, 2.566	
	Stockport	0.377	0.130, 1.097	
	West Pennine	1.203	0.289, 4.999	
	Wigan and Bolton	0.367	0.117, 1.145	
	Wirral	0.562	0.134, 2.354	
	Bro Taf	198.4	0, ∞	
	Dyfed Powys	0.449	0.146, 1.383	
	Gwent	0.206	0.053, 0.804	
	Lechyd Morgannwg	0.366	0.078, 1.720	
	North Wales	0.546	0.207, 1.443	
Age		0.994 per year	0.982, 1.006	0.314
Travel outside the UK	Y	5.650	2.861, 11.160	<0.001
	N	1.000		
Contact with another person with diarrhoea	Y	4.614	2.449, 8.691	<0.001
	N	1.000		
Touch any cattle	Y	3.876	1.4196, 10.04	0.003
	N	1.000		
Usually wash before eating raw fruit and vegetables	Always	1.000		0.108
	Usually	0.966	0.605, 1.543	
	Sometimes	0.746	0.436, 1.274	
	Never	2.478	0.965, 6.362	
Toileting contact with child under 5 years of age	Y	1.851	1.079, 3.175	0.025
	N	1.000		
Number of glasses of unboiled water drunk at home		1.135 per glass	1.019, 1.265	0.019
Eat ice cream	Y	0.472	0.299, 0.746	0.001
	N	1.000		
Eat raw vegetables	Y	0.532	0.346, 0.820	0.004
	N	1.000		
Eat tomatoes	Y	0.616	0.392, 0.969	0.035
	N	1.000		

n.e. not estimable

The model in table 5 shows the final positive only model fror cases of genotype 1 and was estimated from 463 observations. Health Authority of residence, travel outside the UK, nappy changing contact with a child under 5 years and contact with another person with diarrhoea were strongly associated with illness ($P<0.01$), whilst frequency of washing raw vegetables was moderately significant ($P<0.05$). In the positive and negative model, travel outside the UK and nappy changing contact

remained strongly positive. Sleeping on the ground, the number of people living with the person, eating fresh fruit and the likelihood of washing fresh fruit and vegetables were negatively associated with risk. Contact with another person with diarrhoea was rejected from the model.

Table 5. Final multivariable model (positively associated variables only in initial multivariable model) – genotype 1. Estimated from 463 observations

		Adjusted Odds Ratio	95% Confidence Interval	p value
Health Authority	Bury and Rochdale	1.000		<0.001
	East Lancashire	0.114	0.024, 0.540	
	Liverpool	n.e.	n.e.	
	Manchester	0.891	0.273, 2.906	
	Morecambe Bay	0.002	0, ∞	
	North West Lancashire	0.228	0.056, 0.926	
	North Cheshire	0.443	0.064, 3.050	
	Salford and Trafford	0.210	0.017, 2.597	
	Sefton	n.e.	n.e.	
	South Cheshire	0.099	0.021, 0.469	
	South Lancashire	n.e.	n.e.	
	St Helens and Knowsley	0.389	0.025, 6.102	
	Stockport	1.635	0.517, 5.169	
	West Pennine	2.977	0.749, 11.83	
	Wigan and Bolton	0.150	0.016, 1.429	
	Wirral	0.001	0, ∞	
	Bro Taf	n.e.	n.e.	
	Dyfed Powys	0.152	0.025, 0.914	
	Gwent	0.325	0.067, 1.577	
	Lechyd Morgannwg	1.344	0.281, 6.423	
	North Wales	0.728	0.233, 2.273	
Age		0.990 per year	0.976, 1.004	0.144
Travel outside the UK	Y	10.070	4.392, 23.080	<0.001
	N	1.000		
Spend time sleeping or sitting outside on the ground	Y	0.345	0.103, 1.151	0.065
	N	1.000		
Nappy changing contact with a child under 5 years of age	Y	2.931	1.435, 5.989	0.004
	N	1.000		
Contact with another person with diarrhoea	Y	3.886	1.749, 8.636	0.001
	N	1.000		
Usually wash before eating raw fruit and vegetables	Always	1.000		0.018
	Usually	0.373	0.182, 0.763	
	Sometimes	0.858	0.414, 1.777	
	Never	1.601	0.502, 5.106	

n.e. not estimable

Table 6. Final multivariable model (positively and negatively associated variables in initial multivariable model)– genotype 1. Estimated from 433 observations.

		Adjusted Odds Ratio	95% Confidence Interval	p value
Health Authority	Bury and Rochdale	1.000		<0.001
	East Lancashire	0.030	0.003, 0.335	
	Liverpool	n.e.	n.e.	
	Manchester	0.781	0.206, 2.960	
	Morecambe Bay	0.002	0, ∞	
	North West Lancashire	0.169	0.034, 0.836	
	North Cheshire	0.277	0.022, 3.516	
	Salford and Trafford	0.229	0.019, 2.734	
	Sefton	n.e.	n.e.	
	South Cheshire	0.072	0.011, 0.456	
	South Lancashire	n.e.	n.e.	
	St Helens and Knowsley	0.398	0.025, 6.396	
	Stockport	2.116	0.573, 7.809	
	West Pennine	5.321	1.098, 25.78	
	Wigan and Bolton	0.169	0.017, 1.685	
	Wirral	0.001	0, ∞	
	Bro Taf	n.e.	n.e.	
	Dyfed Powys	0.126	0.020, 0.809	
	Gwent	0.408	0.065, 2.539	
	Lechyd Morgannwg	1.488	0.273, 8.104	
	North Wales	1.015	0.288, 3.579	
Age		0.997 per year	0.982, 1.012	0.713
Travel outside the UK	Y	6.841	2.622, 17.85	<0.001
	N	1.000		
Number of times swum in a toddler pool		1.258 per time	0.960, 1.649	0.077
Spend time sleeping or sitting outside on the ground	Y	0.241	0.060, 0.968	0.027
	N	1.000		
Nappy changing contact with a child under 5 years of age	Y	3.991	1.848, 8.618	<0.001
	N	1.000		
Usually wash before eating raw fruit and vegetables	Always	1.000		0.022
	Usually	0.347	0.159, 0.757	
	Sometimes	0.967	0.437, 2.139	
	Never	1.337	0.387, 4.629	
Number of people 5 to 15 years of age living with you		0.639 per person	0.413, 0.991	0.037
Eat fresh fruit	Y	0.222	0.058, 0.852	0.027
	N	1.000		

n.e. not estimable

The model in table 7 shows the from cases of genotype 2 in the positive only modeland was estimated from 461 observations. There is only weak evidence that risk of genotype 2 sporadic cryptosporidiosis decreases with age. Touching or handling farm animals is a risk factor. In the positive and negative model eating raw vegetables and eating tomatoes were both strongly negatively associated with illness whilst touching any farm animal was moderately associated with illness.

Table 7. Final multivariable model (positively associated variables only in initial multivariable model) – genotype 2. Estimated from 461 observations

		Adjusted Odds Ratio	95% Confidence Interval	p value
Health Authority	Bury and Rochdale	1.000		<0.001
	East Lancashire	0.328	0.059, 1.816	
	Liverpool	n.e.	n.e.	
	Manchester	0.145	0.014, 1.519	
	Morecambe Bay	0.0006	0, ∞	
	North West Lancashire	0.189	0.029, 1.242	
	North Cheshire	0.001	0, ∞	
	Salford and Trafford	2.036	0.336, 12.360	
	Sefton	n.e.	n.e.	
	South Cheshire	0.311	0.061, 1.584	
	South Lancashire	0.0008	0, ∞	
	St Helens and Knowsley	0.0008	0, ∞	
	Stockport	1.167	0.253, 5.373	
	West Pennine	2.317	0.441, 12.170	
	Wigan and Bolton	0.233	0.022, 2.473	
	Wirral	0.584	0.048, 7.066	
	Bro Taf	n.e.	n.e.	
	Dyfed Powys	1.340	0.295, 6.083	
	Gwent	0.0007	0, ∞	
	Lechyd Morgannwg	0.485	0.043, 5.498	
	North Wales	2.618	0.678, 10.110	
Age		0.985	0.970, 0.9998	0.039
Touch or handle any farm animals	Y	2.474	1.227, 4.986	0.012
	N			

n.e. not estimable

Table 8. Final multivariable model model (positively and negatively associated variables in initial multivariable model)– genotype 2. Estimated from 392 observations

		Adjusted Odds Ratio	95% Confidence Interval	p value
Health Authority	Bury and Rochdale	1.000		<0.001
	East Lancashire	0.296	0.039, 2.249	
	Liverpool	n.e.	n.e.	
	Manchester	0.0001	0, ∞	
	Morecambe Bay	0.0002	0, ∞	
	North West Lancashire	0.118	0.009, 1.552	
	North Cheshire	0.0006	0, ∞	
	Salford and Trafford	0.745	0.050, 11.17	
	Sefton	n.e.	n.e.	
	South Cheshire	0.155	0.017, 1.367	
	South Lancashire	0.00005	0, ∞	
	St Helens and Knowsley	0.0002	0, ∞	
	Stockport	0.981	0.136, 7.082	
	West Pennine	2.390	0.308, 18.56	
	Wigan and Bolton	0.0002	0, ∞	
	Wirral	0.425	0.028, 6.360	
	Bro Taf	n.e.	n.e.	
	Dyfed Powys	1.239	0.186, 8.260	
	Gwent	0.0001	0, ∞	
	Lechyd Morgannwg	0.643	0.043, 9.545	
	North Wales	2.260	0.398, 12.83	
Age		0.993	0.972, 1.015	0.530
Touch or handle any farm animals	Y	2.653	1.113, 6.323	0.028
	N			
Eat tomatoes	Y	0.317	0.140, 0.719	0.005
	N	1.000		
Eat raw vegetables	Y	0.222	0.086, 0.572	0.001
	N	1.000		

n.e. not estimable

In addition to asking questions about possible risk factors, the questionnaire asked both cases and controls (or their parents or guardians) if they had heard about *Cryptosporidium* before receiving the questionnaire. Not surprisingly cases were more likely to have heard of *Cryptosporidium* before receiving the questionnaire than controls (56% *vs* 28%; p<0.0001). The source of people's information is shown in table 9. Several respondents indicated finding out from more than one source. For cases, the most common source of their information came from the result of their stool test, their GPs or nurse or from the Environmental Health Officer who visited. Where controls had heard about *Cryptosporidium*, they are most likely to have picked up their information from "other sources" usually because of their occupation, or a past infection in themselves or family members. In addition, the newspapers and television were cited by more than 20%.

Table 9. Prior knowledge about Cryptosporidium

	Cases (n=232)	**Controls (n=111)**
Heard of Cryptosporidium from the results of your stool test	150 (64.7%)	1 (0.9%)
Heard of Cryptosporidium from television	19 (8.2%)	26 (23.4%)
Heard of Cryptosporidium from doctor or nurse	47 (20.3%)	19 (17.1%)
Heard of Cryptosporidium from the newspaper	17 (7.3%)	32 (28.8%)
Heard of Cryptosporidium from a magazine	4 (1.7%)	8 (7.2%)
Heard of Cryptosporidium from a health leaflet	12 (5.2%)	9 (8.1%)
Heard of Cryptosporidium from friends or relatives	21 (9.1%)	14 (12.6%)
Heard of Cryptosporidium from the internet	13 (5.6%)	0
Heard of Cryptosporidium from a pharmacy	1 (0.4%)	1 (0.9%)
Heard of Cryptosporidium from a visit from an Environmental Health Officer	77 (33.2%)	3 (2.7%)
Heard of Cryptosporidium from some other source (usually because of their occupation or a past infection in themselves or family)	39 (16.8%)	38 (34.2%)

Discussion

There have been very few prospective case control studies examining the risk factors of sporadic *Cryptosporidium* infection. Indeed, only one sizeable case control study has been reported to date from a developed nation (Robertson et al, 2002). However, our study is the first prospective epidemiological study of sporadic cryptosporidiosis that has been able to separately investigate risk factors for *C. parvum* genotype 1 and genotype 2 infections.

In this study we analysed a large number of variables, indeed the number of variables associated with risk of illness at the 0.20 level was so large that not all could be included the initial models within the computer package used for these analyses. We present in this report two approaches to dealing with this large number of variables, the first was to present a model with only positively associated variables and the second was to add negatively associated variables as and when space became available due to removal of existing variable as discussed above. An advantage of the models with only positively associated variables is that they will be modelled on larger numbers of cases and controls and so are likely to be more robust. However, they may suffer from confounding from variables not included in the model. In this analysis we present the models determined in both ways (positive and mixed). Clearly we can be more confident about positively associated risk factors that achieve higher levels of significance ($p<0.01$) in both models. Negatively associated variables will, of course only appear in the mixed model. Conclusions based on variables that achieve lower levels of significance in only one model are less robust.

Analysis of all cases

The risk factors identified in the combined analysis are, in general, not surprising. The main risk factors identified are broadly similar to what would have been predicted from an analysis of outbreaks and similar to those identified by Robertson and colleagues (2002); travel abroad, contact with a case and touching cattle. This was found to be the case in both models (positive and mixed). In the model with both positive and negative risk factors strongly significant negative factors were eating ice

cream and raw vegetables. Frequency of washing raw fruit and vegetables and having a medical condition affecting immunity were significant in the positive model only.

Factors significant at the 0.05 level in the positive model were age and the number of times swum in a toddler pool. In the mixed model, “toileting contact with a child under 5 years of age” and “number of glasses of unboiled water drunk at home” were positively associated with illness at the 0.05 level and eating tomatoes negatively associated at this level.

Significant differences in the risk of sporadic cryptosporidiosis were found between health authorities, but this was due to just six of the health authorities, four in North West England and two in Wales that appeared to have significantly lower risk of infection than Bury and Rochdale (used for the comparator because for alphabetic reasons only). Differences in the timeliness of reporting between health authorities may have had a slight impact upon results given that case notifications exceeding four weeks were excluded from the study. However consistent differences in the rate of cryptosporidiosis between health authorities, particularly within the North West of England have previously been documented (tables 10 and 11). This is further investigated in a supplemental report. It should be noted that these tables represent cases reported to CDSC and not all laboratories were reporting throughout the periods covered by the tables

Table 10. Annual incidence rate per 100,000 population/year for each Health Authority in the North West Region.

	1990	**1991**	**1992**	**1993**	**1994**	**1995**	**1996**	**1997**	**1998**	**1999**
BURY & ROCHDALE	19.6	18.5	29.3	25.8	14.9	27.2	17.7	23.6	16.4	22.6
EAST LANCASHIRE	7.8	14	28.7	21.8	20.9	20.3	12.9	19.9	13.3	8
LIVERPOOL	3.5	5	3.3	2.1	2.1	2.1	4.7	0.6	1.3	0.2
MANCHESTER	25.1	42.4	43.9	29.4	18.3	6.2	28.3	28.6	14.2	15.1
MORECAMBE BAY	8.6	18.8	37.9	19.9	15	17.5	16.2	23.6	17.8	9.7
NORTH CHESHIRE	1.5	7	10	8.4	3.2	1.9	4.9	0.4	3.2	1.5
NW LANCASHIRE	34.2	34.5	56.1	37.2	50.6	62.7	24	62.5	42.6	56.1
SALFORD & TRAFFORD	6.3	15.2	15.7	16.1	8.5	11.4	10.5	23.9	9.2	20.1
SEFTON	2.7	4.1	5.1	4.4	7.2	4.1	3.1	7.2	2.1	2.4
SOUTH CHESHIRE	6	4.6	7.6	4.8	4.4	11.5	7.2	9	9.6	7.3
SOUTH LANCASHIRE	1	0.6	6.5	11.3	2.3	4.5	6.1	9.1	15.8	45.6
ST. HELENS & KNOWSLEY	0	1.1	0	0.2	0.7	0	0.2	2	2	0.4
STOCKPORT	2.2	3.9	4.4	12.2	5.6	6.1	1.7	8.9	10	21.2
WEST PENNINE	6.5	4	11.9	3.6	4	4.2	4.3	7	13.2	16.4
WIGAN & BOLTON	13.5	14.6	24.1	17.5	17.9	10.8	15.5	26.9	20.9	13.6
WIRRAL	0	0	0	0	0	0	0	0	0.9	1.2
North West Region	8.6	11.7	17.5	12.9	10.6	11.3	10	15.4	11.7	13.6
England & Wales	9.6	10.5	10.6	9.9	9.1	11.6	7.5	8.8	7.6	9.7

Table 11. Laboratory reports of Cryptosporidium to CDSC(Wales) by DHA, rates per 100,000 population*: 1990-1999

	1990	1991	1992	1993	1994	1995	1996	1997	1998	1999
Bro Taf	5.3	7.8	9.2	12.3	12.7	16.9	3.5	3.4	4.3	4.2
Dyfed Powys	5.4	14.2	21.5	15.2	15.7	19.4	12.9	16.9	16.9	16.1
Gwent	3.1	7.2	4.5	0.5	3.2	1.8	2.0	2.7	4.0	5.0
Morgannwg	1.6	1.6	0.6	0.8	1.8	5.2	3.2	2.8	4.6	2.8
North Wales	16.6	25.4	21.3	22.5	21.8	19.0	21.6	16.3	18.9	28.8
Annual Mean (Wales)	6.8	11.6	11.6	11.3	11.6	13.0	8.8	8.3	9.7	11.6

* ONS mid-1998 population estimates used to calculate rates

Source: 1990-1992 data from LabBase**, 1993-1999 data from CoSurv Laboratory Module

** Please note that the 1990-1992 data is by Health Authority of the reporting laboratory not Health Authority of residence

Travel outside of the UK was found to be a significant risk factor. This is consistent with Robertson et al (2002) who identified travel outside Australia as a risk factor. However, they suggested that the odds ratio may be inflated due to ascertainment bias of cases. This problem holds for the present study. GP's may be more likely to request a faecal sample from a patient with diarrhoea who has travelled abroad. In addition, previous research notes that most laboratories in the North West of England and Wales routinely screen for *Cryptosporidium* oocysts if the patient is known to have travelled outside of the UK (Chalmers et al. 1998).

The risk of infection increased significantly upon contact with cattle. Previous research has associated farm animal contact with outbreaks of *Cryptosporidium*, and calf and lamb contact have been identified as risk factors for sporadic infection (Robertson et al, 2002). There have also been several outbreaks associated with farm visits described within the UK (table 1). The risk of contact with other farm animals was not significant, although it is plausible that people in contact with other farm animals were also in contact with cattle. Risk from other farm animals alone would therefore be difficult to ascertain. No significant association was found between ownership or contact with domestic pets and sporadic infection. Although some researchers have suggested pets may present a risk (Casemore et al. 1997), other studies indicate that pets are not a major risk factor for the acquisition of *Cryptosporidium* (Glaser et al. 1998). Indeed, previous research has found various types of domestic animal contact to be protective factors (Robertson et al, 2002).

A further significant risk factor of sporadic cryptosporidiosis was contact with an infected person. Person to person transmission has been identified in outbreak investigations in the UK, and has previously been documented as a risk factor for sporadic cases in Australia (Robertson et al, 2002).

The negative association with consumption of raw vegetables is also consistent with previous studies that have suggested a protective effect from consumption of raw vegetables (Casemore, Wright and Coop 1997; Robertson et al, 2002). Eating a range raw salad foods (green salad other than lettuce, tomatoes, coleslaw, raw vegetables and fresh fruit) were all negatively associated with risk of illness in the single variable but only eating raw vegetables and tomatoes were in the final model. The mechanism

for this negative association is unclear. Whether this represents the effect of immunity through repeat exposure by this route or through another mechanism is unclear (Hunter 2000; Hunter and Quigley 1998).

The negative association with ice cream was unexpected. Unpasteurised milk products have previously been associated with *Cryptosporidium* and were identified as a risk factor for sporadic cases of infection in Adelaide (Robertson et al, 2002). However, in the UK unpasteurised milk is not used in ice-cream production. This association is difficult to explain. We investigated the possibility that this was due to different times of the year that cases and controls were recruited. However, in all but one month controls were more likely to report ice cream consumption than cases. It is notable that a recently published case-control study on risk factors for giardiasis in the South West of England also reported a negative association with ice-cream (Stuart et al. 2003).

Use of a toddler swimming pool was found to be a significant risk factor, specifically, the more frequent the use the higher the risk. Given the age distribution of cases, it is likely that this was the strongest “swimming pool associated” risk factor in this study and so represented the group of variables associated with swimming pool source. The use of a swimming pool has previously been associated with many outbreaks of *Cryptosporidium* in the UK and elsewhere (Rooney et al. In preparation), and use of a toddler pool with sporadic cases (Robertson et al, 2002). The importance of swimming pool exposure as a risk factor for sporadic cryptosporidiosis was suggested by Hunter and Quigley (1998). They demonstrated a protective effect of swimming pool use in an outbreak associated with drinking water and suggested that this was due to immunity from an increased risk of sporadic disease in people who go swimming.

Toddler pools may pose a greater risk of infection due to higher rates of faecal accidents of younger children and an increased likelihood of younger children swallowing pool water. It should be noted that the number of times a person swallowed pool water was not found to be a significant risk factor. However the accuracy of recalling such a measurement could be questioned, particularly in the case of a parent answering for a child. Also, variations in the frequency of swallowing pool

water may be explained by how often a person uses a toddler pool. Higher reported amounts of swallowing pool water may be an indication of higher toddler pool usage.

The risk of developing cryptosporidiosis increased significantly for immunocompromised individuals in the positive risk factor model but not the mixed. Immune system illnesses that depress CD4 counts are well recognised as risk factors of *Cryptosporidium* (Inungu et al. 1998). However, in the few cases that the disease could be identified, these were not those typically associated with increased risk (Hunter and Nichols 2002).

The main difference between previous findings from outbreak studies of *Cryptosporidium* and the present sporadic study concern the consumption of unboiled mains drinking water. Whilst it remains one of the main risk factors in outbreak cases of infection, we could find little evidence of its contribution to sporadic cases. A significant association was found with the number of glasses of unboiled water drunk at home, but only at the 0.05 level in the mixed model. No other mains water-related variable was significant in either the single variable or multivariable analyses. This is consistent with previous case control study findings of sporadic infection and suggests that mains drinking water does not make a significant contribution to the risk of acquiring sporadic *Cryptosporidium* (Robertson et al, 2002). However, in the Australian study the water catchment areas are highly protected with no livestock farming in the catchment. It could be argued that the nature of the water catchment areas in Australia precludes the generalisation of their results to other parts of the world.

Genotype specific analyses

When the data were broken down by genotype, two different models of risk emerged. For both genotypes, the Health Authority variable remained significant, all other risk factors differed. For genotype 1, travelling outside of the UK, being in contact with an infected person and failing to wash fruit or vegetables before consumption remained significant. Changing an infant's nappy was also identified as a significant risk factor. Changing a child's nappy was independent of contact with a case and remained significant, even in the analysis was restricted to cases who had no history of contact

with a case. So changing nappies of an asymptomatic child is a risk factor for type 1 infection. This would suggest that asymptomatic carriage of the human genotype may be common in very young children.

For genotype 2, age and contact with farm animals were found to be the only significant positive risk for to infection. However, in the mixed model eating raw vegetables and tomatoes were both strongly associated with risk of illness. These findings support evidence of the epidemiology of the two genotypes from routine genotyping data. These show that genotype 1 is restricted to causing disease in humans only whilst genotype 2 affects both human and animals (McLauchlin 2000). Also seasonal differences in detection of the two genotypes have been related to in increase in travel associated genotype 1 cases in the late summer and early autumn as people return from their summer holidays (Nichols and McLauchlin 2002).

It should be noted that results from restricting analysis to genotype 1 or 2 had less power than when considering the data as a whole because fewer cases are available for analysis. On the other hand, analyses conducted on populations of cases that contain two pathogens of different epidemiologies may mask genotype specific risk factors.

Regarding clinical details of all cases, symptoms experienced were consistent with what is currently known about the disease. Aside from diarrhoea, the main symptoms experienced were abdominal pain, vomiting and fever. There were no significant differences between the clinical presentations of genotype 1 or 2. Both genotypes showed similar levels of hospital admission, suggesting that disease severity did not differ with type. Duration of illness for all cases was typical of previous reports. Again, no significant differences were found between mean duration for genotype 1 or 2, however the variation of duration for genotype 1 was found to be significantly higher than genotype 2. This suggests that type 1 may be less predictable in terms of duration and more prone to extremes than type 2 infection. Further attention is required to better explain why this may be so.

Other issues

Regarding public knowledge of *Cryptosporidium*, cases unsurprisingly were better informed than controls. Worryingly, a large proportion of cases (or their guardians) claimed not to have heard of *Cryptosporidium* before receiving the questionnaire, despite having had a stool sample taken for testing. This suggests either the results of the samples are not routinely being fed back to the patient or that notifications to patients are delayed. Previous research has identified differences in the procedure of Local Authority Environmental Health departments as to whether *Cryptosporidium* cases are given information about their disease and how to prevent transmission to others (Chalmers et al. 2002). Clearly differences also exist between GP's as to whether patients are informed about their illness and the timeliness of notifications. Of the cases and controls who had heard of *Cryptosporidium,* cases were most likely to have heard from results of their stool test, or from sources related to their recent illness such as doctor, nurse or environmental health officer. Controls were most likely to have picked up their information from other sources such as their occupation or a past infection in themselves or other family members. It is possible that a case's understanding of his/her illness has some influence on how the questionnaire is completed, particularly if a case has strong views regarding cause. Since knowledge will likely influence understanding of illness, it would be interesting to examine what effect knowledge of *Cryptosporidium* and perception of cause has on how a questionnaire investigating risk factors is completed.

In considering the validity of any epidemiological study, one has to consider whether the results and conclusions may be affected by one of several different sources of bias. There are several issues that need to be addressed in this study.

All our cases were taken from reports to Consultants in Communicable Disease Control, usually from laboratories based on positive stool samples. There are a number of different steps that someone has to go through before the infection is recorded by the CCDC (Chalmers et al. 2002; Wheeler et al. 1999). This is known as the reporting pyramid. The first stage is for someone to become infected with the pathogen, this person may or may not then become ill, he/she may or may not then present to the General Practitioner who may or may not send a stool sample to the laboratory that may or may not look for *Cryptosporidium*. Even if the laboratory tests the sample it may miss the diagnosis. Finally the laboratory may not always report to

the CCDC. The factors that influence these various decisions in the process are still not fully understood and it still not absolutely clear how differences in the ascertainment chain may affect the outcome of case-control studies like this. Nevertheless, when considering the conclusions of this report it is well to remember that cases were individuals who became ill, visited their GP and had a specimen taken. There may be geographical variation in the likelihood that patients attend their GP and in how likely specimens are taken and results reported to CCDCs or CDSC.

The response rate for cases and especially controls is lower than what one would wish, but is in line for this type of study (Stuart et al. 2003). Clearly where ascertainment is less than 100% there is always the potential for non-response bias to affect the findings of the study. It is known that response rates are lower in the very young and old, in unmarried adults and among people who are unemployed or in the lower socio-economic groups (Richiardi, Boffetta and Merletti 2002). This non-response bias can affect the assessment of those risk factors that may be themselves affected by the factors that affect response. The social class distribution of cryptosporidiosis in the UK is still not adequately described. The one where such bias may have had a major impact is in the geographical distribution by health authority. An ecological study based on the enhanced surveillance part of the project will form a subsequent report and this will address the issue of geographical distribution of cases within the two areas of this study.

Recall bias occurs when cases and controls differ in remembering having been exposed to a particular risk factor. Recall bias can have significant impacts on the conclusions that are drawn from epidemiological studies. However, until recently, this source of bias has been considered very rarely in studies of the epidemiology of infectious disease and then only in outbreak settings (Hunter 2000; Hunter and Syed 2002). It is difficult to see how recall bias could have affected the main conclusions drawn from the final models. Nevertheless, people’s views about the causation of their illness have been recorded and will be analysed subsequently.

The other potential source of bias in this study was the dramatic decline in reports of cryptosporidiosis in 2001 throughout the United Kingdom, but especially in the North West Region (Hunter et al. 2003). This decline in incidence was associated with the

epidemic of Foot and Mouth Disease. How the two diseases were related is not clear, though it is thought that the biggest impact would have been driven by control measures that prevented people from gaining access to the countryside. The impact of this change on our conclusions would be that contact with farm animals would be less significant as a result of this change than would have been the case in previous years. Another explanation for this decline is that the spring outbreak of cryptosporidiosis associated with Thirlmere Reservoir seen in previous years did not occur. It could be argued therefore, that our study would underestimate the contribution from drinking water. However, this study was designed to investigate sporadic rather than outbreak-related cryptosporidiosis and any cases identified as being part of an outbreak would have been excluded. Consequently, it is possible that our study underestimates the impact of contact with livestock or animal faeces but not the impact of drinking water.

Conclusions

The main conclusions from this study are that sporadic cryptosporidiosis is strongly associated with travel outside the UK, contact with another person with diarrhoea, touching farm animals, especially cattle, and negatively associated with eating ice cream and eating raw vegetables.

However, the epidemiology of type 1 and type 2 disease appears to be quite different and epidemiological studies that combine the two pathogens risk being misleading. The median age of infection for type 1 was 21 years and that for type 2 was only 9 years.

The main risk factors for type 1 (human) genotypes are travel outside the UK, contact with another person with diarrhoea, changing nappies of children under 5, never washing raw fruit and vegetables before consumption and swimming in a toddler pool.

The only significant risk factor for type 2 (cattle) genotype is contact with farm animals.

Our findings do not suggest that drinking mains tap water is a major risk factor for sporadic cryptosporidiosis in the study area.

References

Anon. (1998) Standard operating procedure for the investigation of specimens other than blood for parasites. PHLS B.SOP 31. Technical Services PHLS HQ London.

Casemore, D.P. (1991) Broadsheet 128: Laboratory methods for diagnosing cryptosporidiosis. *Journal of Clinical Pathology* **44**, 445-451.

Casemore D.P., Wright S.E., Coop R.L. (1997) Cryptosporidiosis – human and animal epidemiology. In: Fayer, R. ed *Cryptosporidium and Cryptosporidiosis.* New York, London, Tokyo: CRC Press, 1997: 65-92.

Chalmers, R.M., Hughes, S., Thomas, A., Woodhouse, S., Thomas, P.D. and Hunter, P. (2002) Laboratory ascertainment of Cryptosporidium and local authority public health policies for the investigation of sporadic cases of cryptosporidiosis in two regions of the United Kingdom. *Communicable Disease and Public Health* **5**: 114-118.

Chen, X.M., Keithly, J.S., Paya, C.V. and LaRusso, N.F. (2002) Cryptosporidiosis. *New England Journal of Medicine* **346**, 1723-1731.

Department of the Environment and Department of Health (1990) *Cryptosporidium* in water supplies: Report of the Group of Experts. London: HMSO.

Department of the Environment and Department of Health (1995) *Cryptosporidium* in water supplies: Second Report of the Group of Experts. London: HMSO.

Department of the Environment, Transport and the Regions, and Department of Health (1998) *Cryptosporidium* in water supplies: Third Report of the Group of Experts. London: HMSO.

Djuretic, T., Wall, P.G., Ryan, M.J., Evans, H.S., Adak, G.K. and Cowden, J.M. (1996) General outbreaks of infectious intestinal disease in England and Wales: 1992 to 1994. *Communicable Disease Report – Review* **6**, R57-R63.

Evans, H.S., Madden, P., Douglas, C., Adak, G.K., O'Brien, S.J., Djuretic, T., Wall, P.G. and Stanwell-Smith, R. (1998) General outbreaks of infectious intestinal disease in England and Wales: 1995 and 1996. *Communicable Disease and Public Health* **1**, 165-171.

Glaser C, Safrin S, Reingold A, Newman TB. (1998) Association between *Cryptosporidium* infection and animal exposure in HIV-infected individuals. *Journal of AIDS* **17**: 79-82.

Hunter, P.R. (1997) *Waterborne disease: epidemiology and ecology*. Wiley, Chichester.

Hunter, P.R. (2000) Modelling the impact of prior immunity, case misclassification and bias on case-control studies in the investigation of outbreaks of cryptosporidiosis. *Epidemiology and Infection* **125**, 713-718.

Hunter, P.R., Chalmers, R.M., Syed, Q., Hughes, L.S., Woodhouse, S. and Swift, L. (2003) Foot and Mouth Disease and Cryptosporidiosis: Possible Interaction between Two Emerging Infectious Diseases. *Emerging Infectious Diseases* **9**, in press.

Hunter, P.R. and Nichols, G. (2002) The epidemiology and clinical features of cryptosporidium infection in immune-compromised patients. *Clinical Microbiology Reviews* **15**, 145-154.

Hunter, P.R. and Quigley, C. (1998) Investigation of an outbreak of cryptosporidiosis associated with treated surface water finds limits to the value of case control studies. *Communicable Disease and Public Health*. **1**, 234-238.

Hunter, P.R. and Syed, Q. (2002) Recall bias in a community survey of self-reported gastroenteritis undertaken during an outbreak of cryptosporidiosis strongly associated with drinking water after much press interest. *Epidemiology and Infection* **128**, 433-438.

Hunter, P.R., Syed, Q. and Naumova, E.N. (2001) Possible undetected outbreaks of cryptosporidiosis in areas of the North West of England supplied by an unfiltered surface water source. *Communicable Disease and Public Health* **4**, 136-138.

Inungu J.N., Morse, A.A. and Gordon, C. (2000) Risk factors, seasonality, and trends of cryptosporidiosis among patients infected with human immunodeficiency virus. *American Journal of Tropical Medicine & Hygiene* **62**(3): 384-7.

Kosek, M., Alcantara, C., Lima, A.A. and Guerrant, R.L. (2001) Cryptosporidiosis: an update. *The Lancet Infectious Diseases* **1**, 262-269.

Linnane, E., Roberts, R.J. and Mannion, P.T. (2002) An outbreak of Salmonella enteritidis phage type 34a infection in primary school children: the use of visual aids and food preferences to overcome recall bias in a case control study. *Epidemiology and Infection* **129**, 35-39.

McLauchlin, J., Amar, C., Pedraza-Diaz, S. and Nichols, G.L. (2000) Molecular epidemiological analysis of Cryptosporidium spp. in the United Kingdom: results of genotyping Cryptosporidium spp. in 1,705 fecal samples from humans and 105 fecal samples from livestock animals. *Journal of Clinical Microbiology* **38**, 3984-3990.

Meinhardt, P.L., Casemore, D.P. and Miller, K.B. (1996) Epidemiologic aspects of human cryptosporidiosis and the role of waterborne transmission. *Epidemiologic Reviews* **18**, 118-136.

Nichols, G. and McLauchlin, J. (2002) Microbiology and the investigation of waterborne outbreaks: The use of *Cryptosporidium* typing in the investigation of waterborne disease. In: Hunter PR, Waite M, Ronchi E (eds). *Drinking Water and Infectious Disease: Establishing the Links*. CRC Press, Bocata Raton, pp 87-95.

Richiardi, L., Boffetta, P. and Merletti, F. (2002) Analysis of nonresponse bias in a population-based case-control study on lung cancer. *Journal of Clinical Epidemiology* **55**, 1033-1040.

Robertson, B., Sinclair, M.I., Forbes, A.B., Veitch, M., Kirk, M., Cunliffe, D., Willis, J. and Fairley, C.K. (2002) Case-control studies of sporadic cryptosporidiosis in Melbourne and Adelaide, Australia. *Epidemiology and Infection* **128**, 419-431.

Rooney, R., Nichols, G.L., Chalmers, R.M., Reacher, M., Stanwell-Smith, R. and Hunter, P.R. (In preparation) Review of outbreaks of infection associated with swimming pools in the UK and Ireland from 1990 to 1999.

Ryley, J.F., Meade, R., Hazelhurst, J. and Robinson, T.E. (1976) Methods in coccidiosis research: separation of oocysts from faeces. *Parasitology* **73**, 311-326.

Spano, F., Putignani, L., McLauchlin, J., Casemore, D.P. and Crisanti, A. (1997) PCR-RFLP analysis of the Cryptosporidium wall protein (COWP) gene discriminates between *C. wrairi* and *C. parvum*, and between *C. parvum* isolates of human and animal origin. *FEMS Microbiology Letters* **150**, 207-217.

Stuart, J.M., Orr, H.J., Warburton, F.G., Jeyakanth, S., Pugh, C., Morris, I., Sarangi, J. and Nichols, G. (2003) Risk Factors for Sporadic Giardiasis: A Case Control Study in Southwestern England. *Emerging Infectious Diseases* **9**, 229-233.

Wheeler, J.G., Sethi, D., Cowden, J.M., Wall, P.G., Rodrigues, L.C., Tompkins, D.S., Hudson, M.J. and Roderick, P.J. (1999) Study of infectious intestinal disease in England: rates in the community, presenting to general practice, and reported to national surveillance. *British Medical Journal* **318**, 1046-1050.

Appendix A. Copies of Questionnaires.

Identification No.

QUESTIONNAIRE FOR SPORADIC CRYPTOSPORIDIOSIS

Adult Cases

Please answer as many of the questions as you can. There are no right or wrong answers. It is OK to answer "don't know"

If you need any help please feel free to contact Miss Sara Hughes on 01244 665305. (Monday – Thursday, 9am – 5pm. Friday 9am – 4.30pm)

Your personal details

1 First Name …… Last Name ……

2 Sex (please tick) ☐ Male ☐ Female

3 Age ……..……...Years

4 Date of Birth ……./……./…….

5 Address……..

Postcode…….. Home Telephone……

6 Main Occupation…… …

7 Address of Workplace……

Postcode …….

8 Country of Birth (please tick)

☐ England ☐ Scotland ☐ Wales ☐ N. Ireland ☐ Irish Republic

☐ Elsewhere (please specify) ……

9 Ethnic Group (please tick)

☐ White ☐ Chinese ☐ Indian ☐ Pakistani ☐ Bangladeshi

☐ Black Caribbean ☐ Black African

☐ Black Other (please specify) ……

☐ Any other ethnic group (please specify) ……..

Your medical details

10 Please give details of your GP

Name ……

Address ……..

…….Telephone No. ……..

11 Do you take regular medication that is known to affect your immunity? (that is your body's ability to fight infections) Please tick

☐ Yes ☐ No ☐ Don't know

If **YES,** please give the name and dose of medication …….

12 Do you have a medical condition that is known to affect your immunity (that is your body's ability to fight infection) Please tick

☐ Yes ☐ No ☐ Don't know

Recent Illness

13 Did you have diarrhoea (3 or more loose stools in 24 hrs) in the 2 weeks before you provided a stool sample? Please tick

☐ Yes ☐ No ☐ Don't know

If **NO**, continue to question 14
If **YES,** on what date did your diarrhoea start? ……/……/……

If **you are better now**, how many days did it last in total? ……..

If **you still have diarrhoea**, for how many days have you had it now? ……..

14 In the two weeks before you provided a stool sample did you have any of the following symptoms? Please tick

Fever	☐ Yes	☐ No	☐ Don't know
Abdominal pain	☐ Yes	☐ No	☐ Don't know
Vomiting	☐ Yes	☐ No	☐ Don't know
Bloody diarrhoea	☐ Yes	☐ No	☐ Don't know
Other	☐ Yes	☐ No	☐ Don't know

If Other please say what ……..……..

15 Were you admitted to hospital because of this illness? Please tick ☐ Yes ☐ No

If **YES**, please give Name of hospital…….

Date of admission …../…../…..

Date of discharge …../…../…..

16 In the 2 weeks before your symptoms started was anyone else who lives in your house ill with diarrhoea? Please tick

☐ Yes ☐ No ☐ Don't know

Background Information

Household details

17 Please tick which best describes where you live:

☐ Private house/flat/apartment ☐ Residential home

☐ Nursing home ☐ Boarding school

☐ Hostel ☐ University/college hall of residence

☐ Other (please state) ……..

18 How many people live with you

Aged over 16 years? ……

Aged 5 – 15 years? ……

Aged less than 5 years? ……...

19 How many other people use the same bathroom as you? Please tick

0	1	2	3	4	5	6	7	8	9	10
☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐

20 How many other people use the same lavatory as you? Please tick

0	1	2	3	4	5	6	7	8	9	10
☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐

21 Which of the following best describes your water supply? Please tick

☐ Mains (please give the company that the bill is paid to)

……..

☐ Borehole ☐ Stream/river ☐ Spring

☐ Sunken well ☐ Dyke ☐ Pond/lake

☐ Ditch ☐ Rainwater tank

☐ Reservoir ☐ Don't know

☐ Other (please specify) ……..

The following questions are about your activities <u>in the 2 weeks before your symptoms started.</u>

Travel

22 In the 2 weeks before your symptoms started did you travel outside the UK? Please tick

☐ Yes ☐ No ☐ Don't know

If **NO,** continue to question 23
If **YES**, please give the countries that you visited ……..

The date you left the UK ……/……/……

The date you returned to the UK ……/……/……..

23 Did you travel within the UK? Please tick ☐ Yes ☐ No

If **YES,** please state the counties/towns that you visited ……

……

Leisure

24 In the 2 weeks before your symptoms started did you do any gardening other than watering? Please tick

☐ Yes ☐ No ☐ Don't know

25 Did you swim in a swimming pool? Please tick

☐ Yes ☐ No ☐ Don't know

If **NO,** continue to question 26.
If **YES,**

a) About how many times did you swim in a pool? Please tick

1	2	3	4	5	6	7	8	9	10	Don't know
☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐

b) Did you swallow water during the swim? Please tick

☐ Yes ☐ No ☐ Don't know

If **YES,** about how many times did you swallow water? Please tick

1	2	3	4	5	6	7	8	9	10	Don't know
☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐

c) Did you use a learner/toddler pool? Please tick

☐ Yes ☐ No ☐ Don't know

If **NO**, continue to question 26
If **YES**,

i) About how many times? Please tick

1	2	3	4	5	6	7	8	9	10	Don't know
☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐

ii) Did you swallow water during swimming? Please tick

☐ Yes ☐ No ☐ Don't know

iii) About how many times did you swallow water? Please tick

1	2	3	4	5	6	7	8	9	10	Don't know
☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐

26 Did you swallow water during any of the following activities? (please tick)

☐ Swimming in sea	☐ Swimming in river/stream	☐ Swimming in lake
☐ Subaqua outdoors	☐ Subaqua in a pool	☐ Canoeing
☐ Sailing	☐ Snorkelling	☐ Surfing
☐ Windsurfing on lake	☐ Windsurfing on sea	☐ Working in water
☐ Don't know	☐ Other (please say what) ……	

27 Did you spend time sitting or sleeping outside on the ground? (eg. camping, attending rock festival etc) Please tick

☐ Yes ☐ No ☐ Don't know

Contact with Pets

28 In the 2 weeks before your symptoms started were there any domestic pets living in your home? Please tick

☐ Yes ☐ No ☐ Don't know

If **NO**, continue to question 29.
If **YES,**

a) Did you touch or handle any of these pets? Please tick

☐ Yes ☐ No ☐ Don't know

b) Please tick the type of pet/s

☐ cat ☐ dog ☐ hamster ☐ gerbil

☐ bird ☐ reptile ☐ guinea pig ☐ ferret

☐ rat or mouse ☐ other (please say what) ……..

c) Were any of the pets under 6 months old? Please tick

☐ Yes ☐ No ☐ Don't know

If **YES**, please say which ……

d) Did you touch or handle any other pets? Please tick

☐ Yes ☐ No ☐ Don't know

If **YES**, please say which pets and how many ……

Contact with farm animals

29 In the 2 weeks before your symptoms started did you touch or handle any farm animals including birds? Please tick

☐ Yes ☐ No ☐ Don't know

If **YES**, please say what type of farm animal ……

30 Did you touch or handle any zoo animals? Please tick

☐ Yes ☐ No ☐ Don't know

If **YES**, please say what type of zoo animal ……..

31 Did you touch or handle any wild animal? Please tick

☐ Yes ☐ No ☐ Don't know

If **YES**, please say what type of wild animal……

32 Did you touch or handle any animal manure or bird droppings? Please tick

☐ Yes ☐ No ☐ Don't know

Daily activities

33 In the 2 weeks before your symptoms started did you have any of the following contacts with a child under 5 years of age? Please tick

☐ Toileting ☐ Nappy changing

☐ Feeding ☐ Bathing/washing

34 Did you provide close personal care (e.g. toileting, bathing, changing or feeding) for an adult or older child? Please tick

☐ Yes ☐ No ☐ Don't know

35 Did you have contact with another person who was ill with diarrhoea? Please tick

☐ Yes ☐ No ☐ Don't know

Drinking water

36 In the 2 weeks before your symptoms started did you drink **unboiled** tap water or any drinks containing **unboiled** tap water at home? Please tick

☐ Yes ☐ No ☐ Don't know

If **NO**, continue to question 37
If **YES**,

a) About how many glasses a day? (a glass is about 1/3 pint) Please tick

1	2	3	4	5	6	7	8	9	10	Don't know
☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐

b) Was there any disruption to your water supply at home? Please tick

☐ Yes ☐ No ☐ Don't know

c) Did you notice any of the following? (Please tick)

☐ Discoloration ☐ Altered taste ☐ Loss of water pressure

☐ Other problem (please say what) ……..

37 Did you drink **unboiled** tap water or drinks containing **unboiled** tap water somewhere other than home? Please tick

☐ Yes ☐ No ☐ Don't know

38 Did you use ice cubes? Please tick ☐ Yes ☐ No ☐ Don't know

If **YES**, about how many times? Please tick

1	2	3	4	5	6	7	8	9	10	Don't know
☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐

39 Did you drink any bottled water? Please tick

☐ Yes ☐ No ☐ Don't know

If **NO**, continue to question 40
If **YES**, please give the brand name …….. .. ☐ Brand name unknown

Number of glasses a day ……

Food Consumption

40 In the 2 weeks before your symptoms started how often did you eat the following? (Please tick the box that applies best)

Food	Not at all	1-2 times	3-7 times	Most days	Not sure
Lettuce					
Other green salad					
Tomatoes					
Coleslaw					
Raw vegetables					
Fresh fruit					
Rare steak					
Raw shellfish					
Soft cheese, uncooked					
Hard cheese, uncooked					
Yoghurt					
Ice cream					
Cream					
Freshly pressed apple juice					
Barbecued meat					

41 Did you eat any new or unusual foods? Please tick

☐ Yes ☐ No ☐ Don't know

If **YES**, please say what ……

42 Did you drink **pasteurised** milk? Please tick

☐ Yes ☐ No ☐ Don't know

43 Did you eat cereal with **pasteurised** milk on it? Please tick

☐ Yes ☐ No ☐ Don't know

44 Did you drink **unpasteurised** milk (including goat and sheeps' milk) Please tick

☐ Yes ☐ No ☐ Don't know

45 Did you eat cereal with **unpasteurised** milk on it? (including goat and sheeps' milk) Please tick

☐ Yes ☐ No ☐ Don't know

46 Do you regularly bite your nails or chew fingers? Please tick

☐ Yes ☐ No ☐ Don't know

47 Do you smoke cigarettes or cigars? Please tick

☐ Yes ☐ No ☐ Don't know

48 Do you wash your hands before eating or handling food? Please tick

☐ Always ☐ Usually ☐ Sometimes ☐ Never

This is the end of the questionnaire about the 2 weeks before your symptoms started.

The rest of the questions are about your <u>regular activities at any time.</u>

Drinking water

49 Do you drink **unboiled** tap water or drinks containing **unboiled** tap water at home? Please tick

☐ Yes ☐ No ☐ Don't know

If **YES**, about how many glasses per day? (a glass is about 1/3 pint)

1	2	3	4	5	6	7	8	9	10	Don't know
☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐

50 Do you use a water filter at home? Please tick

☐ Yes ☐ No ☐ Don't know

51 Do you drink **unboiled** tap water or drinks containing **unboiled** tap water somewhere other than at home? Please tick

☐ Yes ☐ No ☐ Don't know

If **YES**, about how many glasses per day? (a glass is about $^{1}/_{3}$ pint) Please tick

1	2	3	4	5	6	7	8	9	10	Don't know
☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐

52 If you eat raw fruit and vegetables, do you wash them before eating? Please tick

☐ Always ☐ Usually ☐ Sometimes ☐ Never

Your understanding of your illness

53 What do you think may have caused your illness? Please say briefly in your own words

……

……

……

……

54 How sure are you of the above statement? Please tick the number that fits best, with 1 being not very sure and 10 being absolutely certain.

(Not very sure) (Certain)

→

1	2	3	4	5	6	7	8	9	10
☐	☐	☐	☐	☐	☐	☐	☐	☐	☐

55 Before receiving this questionnaire had you heard of Cryptosporidium? Please tick

☐ Yes ☐ No ☐ Don't know

If **YES**, where from? Please tick

☐ results of your stool test ☐ television ☐ doctor/nurse

☐ newspaper ☐ magazine ☐ health leaflet

☐ friends/relatives ☐ the internet ☐ pharmacy

☐ Environmental Health Officer

☐ other (please say where) ……

56 Have you been visited by an Environmental Health Officer? Please tick

☐ Yes ☐ No

57 We may want to write to a few people again at a later date with a similar questionnaire. Would you be happy for us to contact you again? Please tick

☐ Yes ☐ No

This is the end of the questions. Many thanks for your help in completing this questionnaire.

Please sign and date below.

Signature…….. Date……

Appendix B. Copies of Information leaflets

Cryptosporidiosis Study in Wales and the North West of England

You are being invited to fill in a questionnaire for a research study. Before you decide to take part it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully and discuss it with friends, relatives and your GP if you wish. Ask us if there is anything that is not clear or if you would like more information. Take time to decide whether or not you wish to take part.

What is the purpose of the study?
The study is being done to find out more about a common gastro-intestinal illness called *cryptosporidiosis*. We want to find out why some people catch it and others don't so that we can help to prevent it.

Why is cryptosporidiosis important?
Because it affects a lot of people and sometimes causes outbreaks in an area. It often affects children. Although most people get better quickly, a few can stay ill for longer. It can be a serious and sometimes life-threatening illness for people who can't easily fight off infections.

Why have I been chosen?
We are sending questionnaires to people who have been ill with *cryptosporidiosis* recently and also to people of the same sex and age who haven't been ill. We monitor routine lab reports and have written to you because your recent stool test was positive for cryptosporidiosis. By comparing answers from the ill people with answers from the people who haven't been ill, we hope to learn more about the reasons why some people get this illness and others don't.

Do I have to take part?
It is up to you to decide whether or not to take part. If you take part you are still free to withdraw at any time and without giving a reason. If you choose not to take part it will not affect your medical care or legal rights.

What do I have to do if I take part?
Fill in the questionnaire and send it back to us in the pre-paid envelope. There are instructions on the questionnaire. Don't worry if you can't answer some of the questions - it is OK to put "don't know". If we do not get the questionnaire back after 2 weeks we will write to you again asking you to fill in the questionnaire. If you do not send it back to us that time, we will not write to you again.

Will it be confidential?
Yes. The information that you give us will be treated with strict confidence in the same way as other medical information. Only members of the small research team will know your personal details. They are doctors and essential support staff who are used to handling confidential information. When the results are analysed and reports written on the findings of the study, all names and personal details will be removed so you cannot be identified.

What will happen to the results of the research study?
We hope that the results will help us to make recommendations to prevent people getting ill with *cryptosporidiosis*. The results will be put into reports and may be published in medical or scientific journals and presented at scientific conferences. In this way, other doctors and scientists can share the information and make comments on it. We may also be able to make recommendations to health and other organisations and the public about how to prevent *cryptosporidiosis*.

Who is organising and funding the research?
The research is being organised and carried out by a small team of people working at the Communicable Disease Surveillance Centre (North West) and the Cryptosporidiosis Reference Unit, Public Health Laboratory Service, Swansea.

The funding is from:

NHS Executive North West
The Drinking Water Inspectorate
North West Water

The researchers are carrying out the work as part of their routine workload and receive no extra payment for it.

Who has reviewed the study?
The study has been reviewed and approved by the North West Multi-centre Research Ethics Committee.

If you have any questions or comments you can call 01244 665305 (9am to 5pm Monday to Thursday, 9am to 4:30pm Friday) and speak to Miss Sara Hughes.

Cryptosporidiosis Study in Wales and the North West of England

You are being invited to fill in a questionnaire about your child for a research study. Before you decide whether your child should take part, it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully and discuss it with your child, friends, relatives and your GP if you wish. Ask us if there is anything that is not clear or if you would like more information. Take time to decide whether or not you wish your child to take part.

What is the purpose of the study?
The study is being done to find out more about a common gastro-intestinal illness called *cryptosporidiosis*. We want to find out why some people catch it and others don't so that we can help to prevent it.

Why is cryptosporidiosis important?
Because it affects a lot of people and sometimes causes outbreaks in an area. It often affects children. Although most people get better quickly, a few can stay ill for longer. It can be a serious and sometimes life-threatening illness for people who can't easily fight off infections.

Why have I been chosen?
We are sending questionnaires to children who have been ill with *cryptosporidiosis* recently and also to children of the same sex and age who haven't been ill. We monitor routine lab reports and have written to you because your child's recent stool test was positive for *cryptosporidiosis*. By comparing answers from the ill children with answers from the children who haven't been ill, we hope to learn more about the reasons why some people get this illness and others don't.

Does my child have to take part?
It is up to you and your child to decide whether or not to take part. If you take part you are still free to withdraw at any time and without giving a reason. If you choose not to take part it will not affect your or your child's medical care or legal rights.

What do I have to do if my child takes part?
Fill in the questionnaire for your child and send it back to us in the pre-paid envelope. There are instructions on the questionnaire. Don't worry if you can't answer some of the questions - it is OK to put "don't know". If we do not get the questionnaire back after 2 weeks we will write to you again asking you to fill in

the questionnaire. If you do not send it back to us that time, we will not write to you again.

Will it be confidential?
Yes. The information that you give us will be treated with strict confidence in the same way as other medical information. Only members of the small research team will know your child's personal details. They are doctors and essential support staff who are used to handling confidential information. When the results are analysed and reports written on the findings of the study, all names and personal details will be removed so your child cannot be identified.

What will happen to the results of the research study?
We hope that the results will help us to make recommendations to prevent people getting ill with *cryptosporidiosis*. The results will be put into reports and may be published in medical or scientific journals and presented at scientific conferences. In this way, other doctors and scientists can share the information and make comments on it. We may also be able to make recommendations to health and other organisations and the public about how to prevent *cryptosporidiosis*.

Who is organising and funding the research?
The research is being organised and carried out by a small team of people working at the Communicable Disease Surveillance Centre (North West) and the Cryptosporidiosis Reference Unit, Public Health Laboratory Service, Swansea.

The funding is from:

NHS Executive North West
The Drinking Water Inspectorate
North West Water

The researchers are carrying out the work as part of their routine workload and receive no extra payment for it.

Who has reviewed the study?
The study has been reviewed and approved by the North West Multi-centre Research Ethics Committee.

If you have any questions or comments you can call 01244 665305 (9am to 5pm Monday to Thursday, 9am to 4:30pm Friday) and speak to Miss Sara Hughes.

Cryptosporidiosis Study in Wales and the North West of England

You are being invited to fill in a questionnaire for a research study. Before you decide to take part it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully and discuss it with friends, relatives and your GP if you wish. Ask us if there is anything that is not clear or if you would like more information. Take time to decide whether or not you wish to take part.

What is the purpose of the study?
The study is being done to find out more about a common gastro-intestinal illness called *cryptosporidiosis*. We want to find out why some people catch it and others don't so that we can help to prevent it.

Why is cryptosporidiosis important?
Because it affects a lot of people and sometimes causes outbreaks in an area. It often affects children. Although most people get better quickly, a few can stay ill for longer. It can be a serious and sometimes life-threatening illness for people who can't easily fight off infections.

Why have I been chosen?
We are sending questionnaires to people who have been ill with *cryptosporidiosis* recently and also to people of the same sex and age who haven't been ill. Your name has been chosen at random from a list of people registered with GP's, because you are the same sex and roughly the same age as someone who has been ill. By comparing answers from the ill people with answers from the people who haven't been ill, we hope to learn more about the reasons why some people get this illness and others don't.

Do I have to take part?
It is up to you to decide whether or not to take part. If you take part you are still free to withdraw at any time and without giving a reason. If you choose not to take part it will not affect your medical care or legal rights.

What do I have to do if I take part?
Fill in the questionnaire and send it back to us in the pre-paid envelope. There are instructions on the questionnaire. Don't worry if you can't answer some of the questions - it is OK to put "don't know". If we do not get the questionnaire back after 2 weeks we will write to you again asking you to fill in the questionnaire. If you do not send it back to us that time, we will not write to you again.

Will it be confidential?
Yes. The information that you give us will be treated with strict confidence in the same way as other medical information. Only members of the small research team will know your personal details. They are doctors and essential support staff who are used to handling confidential information. When the results are analysed and reports written on the findings of the study, all names and personal details will be removed so you cannot be identified.

What will happen to the results of the research study?
We hope that the results will help us to make recommendations to prevent people getting ill with *cryptosporidiosis*. The results will be put into reports and may be published in medical or scientific journals and presented at scientific conferences. In this way, other doctors and scientists can share the information and make comments on it. We may also be able to make recommendations to health and other organisations and the public about how to prevent *cryptosporidiosis*.

Who is organising and funding the research?
The research is being organised and carried out by a small team of people working at the Communicable Disease Surveillance Centre (North West) and the Cryptosporidiosis Reference Unit, Public Health Laboratory Service, Swansea.

The funding is from:

NHS Executive North West
The Drinking Water Inspectorate
North West Water

The researchers are carrying out the work as part of their routine workload and receive no extra payment for it.

Who has reviewed the study?
The study has been reviewed and approved by the North West Multi-centre Research Ethics Committee.

If you have any questions or comments you can call 01244 665305 (9am to 5pm Monday to Thursday, 9am to 4:30pm Friday) and speak to Miss Sara Hughes.

Cryptosporidiosis Study in Wales and the North West of England

You are being invited to fill in a questionnaire about your child for a research study. Before you decide whether your child should take part, it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully and discuss it with your child, friends, relatives and your GP if you wish. Ask us if there is anything that is not clear or if you would like more information. Take time to decide whether or not you wish your child to take part.

What is the purpose of the study?
The study is being done to find out more about a common gastro-intestinal illness called *cryptosporidiosis*. We want to find out why some people catch it and others don't so that we can help to prevent it.

Why is cryptosporidiosis important?
Because it affects a lot of people and sometimes causes outbreaks in an area. It often affects children. Although most people get better quickly, a few can stay ill for longer. It can be a serious and sometimes life-threatening illness for people who can't easily fight off infections.

Why have I been chosen?
We are sending questionnaires to children who have been ill with *cryptosporidiosis* recently and also to children of the same sex and age who haven't been ill. Your child's name has been chosen at random from a list of children registered with GPs, because he or she is the same sex and roughly the same age as a child who has been ill. By comparing the answers from the ill children with the answers from the children who haven't been ill, we hope to learn more about the reasons why some children get this illness and others don't.

Does my child have to take part?
It is up to you and your child to decide whether or not to take part. If you take part you are still free to withdraw at any time and without giving a reason. If you choose not to take part it will not affect your or your child's medical care or legal rights.

What do I have to do if my child takes part?
Fill in the questionnaire for your child and send it back to us in the pre-paid envelope. There are instructions on the questionnaire. Don't worry if you can't answer some of the questions - it is OK to put "don't know". If we do not get the

questionnaire back after 2 weeks we will write to you again asking you to fill in the questionnaire. If you do not send it back to us that time, we will not write to you again.

Will it be confidential?
Yes. The information that you give us will be treated with strict confidence in the same way as other medical information. Only members of the small research team will know your child's personal details. They are doctors and essential support staff who are used to handling confidential information. When the results are analysed and reports written on the findings of the study, all names and personal details will be removed so your child cannot be identified.

What will happen to the results of the research study?
We hope that the results will help us to make recommendations to prevent people getting ill with *cryptosporidiosis*. The results will be put into reports and may be published in medical or scientific journals and presented at scientific conferences. In this way, other doctors and scientists can share the information and make comments on it. We may also be able to make recommendations to health and other organisations and the public about how to prevent *cryptosporidiosis*.

Who is organising and funding the research?
The research is being organised and carried out by a small team of people working at the Communicable Disease Surveillance Centre (North West) and the Cryptosporidiosis Reference Unit, Public Health Laboratory Service, Swansea.

The funding is from:

NHS Executive North West
The Drinking Water Inspectorate
North West Water

The researchers are carrying out the work as part of their routine workload and receive no extra payment for it.

Who has reviewed the study?
The study has been reviewed and approved by the North West Multi-centre Research Ethics Committee.

If you have any questions or comments you can call 01244 665305 (9am to 5pm Monday to Thursday, 9am to 4:30pm Friday) and speak to Miss Sara Hughes.

APPENDIX C - Single Variable Analysis (c^2 Test or Fisher's Exact Test)

		Cases	Controls	Odds Ratio	95% CIs	p-value
Sex	M	204	190	1.02	0.76, 1.35	0.967
	F	222	210			
Place of child attendance	Nursery	48	34			0.128
	Playgroup	16	16	0.71	0.31, 1.61	
	School	119	153	0.55	0.33, 0.91	
	Other	10	10	0.71	0.27, 1.89	
Country of birth	England	324	320			0.390
	Wales	89	69	1.185	0.36, 3.92	
	Other than England & Wales	6	5	1.274	0.90, 1.81	
Ethnic group	White	403	381	0.95	0.35, 2.59	0.899
	Non-white	10	9			
Take regular medication known to affect immunity	Y	11	11	0.96	0.38, 2.42	0.909
	N	392	375			
Have a medical condition known to affect immunity	Y	17	3	5.67	1.54, 24.78	0.004
	N	376	376			
Anyone else in previous 2 weeks in house ill with diarrhoea	Y	83	28	3.20	1.98, 5.20	<0.001
	N	334	361			
Living place	Private house/flat	393	370			0.773
	Residential home	15	14	1.009	0.48, 2.12	
	Other	18	13	1.303	0.63, 2.71	
Number of people over 16 years living with you	0	19	17			0.879[1]
	1	126	110	1.02	0.48, 2.18	
	2	204	196	0.93	0.45, 1.94	
	3	35	31	1.01	0.41, 2.47	
	4	16	10	1.43	0.46, 4.54	
	5 or more	3	3	0.89	0.11, 7.63	
Number of people 5-15 years living with you	0	115	63			<0.001[1]
	1	115	103	0.61	0.40, 0.94	
	2	39	53	0.40	0.23, 0.70	
	3	12	11	0.60	0.23, 1.59	
	4	2	4	0.27	0.02, 1.99	
	5 or more	0	1	0.00	0.00, 21.70	
Number of people less than 5 years living with you	0	137	98			0.845[1]
	1	98	67	1.05	0.68, 1.60	
	2	14	8	1.25	0.47, 3.41	
	3	0	1	0.00	0.00, 28.18	
Number of people sharing the same bathroom	0	25	27			0.764[1]
	1	80	75	1.15	0.59, 2.26	
	2	91	92	1.07	0.55, 2.07	
	3	143	120	1.29	0.68, 2.43	
	4	50	51	1.06	0.51, 2.18	
	5 or more	35	33	1.15	0.52, 2.52	
Number of people sharing the same lavatory	0	41	39			0.964[1]
	1	76	74	0.98	0.55, 1.74	
	2	89	87	0.97	0.55, 1.71	
	3	128	111	1.10	0.64, 1.88	
	4	40	46	0.83	0.43, 1.59	
	5 or more	36	34	1.01	0.50, 2.01	

[1] Chi-Squared Test for Trend

		Cases	Controls	Odds Ratio	95% CIs	p-value
Water supply	Mains Not mains	398 16	374 13	0.86	0.38, 1.94	0.847
Travel outside UK	Y N	99 327	24 376	4.74	2.89, 7.85	<0.001
Travel within the UK	Y N	106 285	122 241	0.73	0.53, 1.02	0.063
Gardening other than watering	Y N	62 345	90 306	0.61	0.42, 0.89	0.009
Swim in a swimming pool	Y N	172 252	147 248	1.15	0.86, 1.55	0.362
Number of times swum in a swimming pool	0 1 2 3 4 5 or more	252 38 39 18 13 53	248 46 52 17 11 18	 0.81 0.74 1.04 1.16 2.90	 0.50, 1.33 0.46, 1.19 0.50, 2.18 0.48, 2.84 1.60, 5.29	<0.001[1]
Swallow pool water	Y N	88 277	78 271	1.10	0.77, 1.59	0.640
Number of times swallow pool water	0 1 2 3 4 5 or more	277 4 13 6 8 10	271 7 22 3 4 6	 0.56 0.58 1.96 1.96 1.63	 0.12, 2.23 0.27, 1.23 0.41, 12.20 0.52, 8.97 0.53, 5.53	0.498[1]
Use a toddler pool	Y N	76 345	50 344	1.52	1.01, 2.28	0.043
Number of times swum in toddler pool	0 1 2 3 4 5 or more	345 16 21 5 6 18	344 16 22 4 3 1	 1.00 0.95 1.25 1.99 17.95	 0.47, 2.14 0.49, 1.84 0.27, 6.33 0.42, 12.41 2.80, 749.91	0.001[1]
Swallow toddler pool water	Y N	28 362	26 350	1.04	0.58, 1.89	0.999
Number of times swallow toddler pool water	0 1 2 3 4 5 or more	362 2 3 2 2 3	350 2 10 0 0 1	 0.97 0.29 52505 52505 2.90	 0.07, 13.41 0.05, 1.14 0.60, ∞ 0.60, ∞ 0.23, 152.7	0.523[1]
Swallow water while swimming in the sea	Y N	34 363	19 360	1.77	0.95, 3.32	0.069
Swallow water while swimming in river	Y N	7 390	1 378	6.78	0.83, 149.95	0.069[2]
Swallow water while swimming in lake	Y N	4 393	2 377	1.92	0.30, 15.35	0.687[2]
Swallow water while subaqua outdoors	Y N	2 395	0 379	52105	0.59, ∞	0.500

[1] Chi-Squared Test for Trend
[2] Fisher's Exact Test

		Cases	Controls	Odds Ratio	95% CIs	p-value
Swallow water while subaqua in a pool	Y N	3 394	0 379	67551	1.07, ∞	0.249
Swallow water while canoeing	Y N	1 396	1 378	0.95	0.03, 35.60	1.000[2]
Swallow water while Sailing	Y N	0 397	0 379	n.e.	n.e.	n.e.
Swallow water while snorkelling	Y N	5 392	1 378	4.82	0.54, 111.44	0.218[2]
Swallow water while surfing	Y N	0 397	0 379	n.e.	n.e.	n.e.
Swallow water while windsurfing on lake	Y N	0 397	0 379	n.e.	n.e.	n.e.
Swallow water while windsurfing on sea	Y N	0 397	0 379	n.e.	n.e.	n.e.
Swallow water while working or playing in water	Y N	1 396	2 378	0.48	0.02, 6.81	0.616[2]
Swallow water in any other activity	Y N	21 377	19 361	1.06	0.53, 2.11	0.990
Spend time sitting or sleeping outside on the ground	Y N	46 360	29 361	1.59	0.95, 2.68	0.079
Domestic pets living in home	Y N	207 219	214 186	0.82	0.62, 1.09	0.180
Touch domestic pets	Y N	189 233	194 202	0.84	0.63, 1.13	0.257
Own a cat	Y N	98 328	106 294	0.83	0.59, 1.16	0.279
Own a dog	Y N	12 414	17 383	0.65	0.29, 1.47	0.353
Own a hamster	Y N	12 414	17 383	0.65	0.29, 1.47	0.353
Own a gerbil	Y N	1 425	3 397	0.31	0.01, 3.40	0.359[2]
Own a bird	Y N	18 408	12 388	1.43	0.64, 3.22	0.450
Own a reptile	Y N	1 425	4 396	0.23	0.01, 2.24	0.204[2]
Own a guinea pig	Y N	7 419	11 389	0.59	0.20, 1.68	0.395
Own a ferret	Y N	1 425	0 400	36512	0.16, ∞	1.000[2]
Own a rat or mouse	Y N	3 423	5 395	0.56	0.10, 2.73	0.494[2]
Own some other pet	Y N	28 398	38 362	0.67	0.39, 1.15	0.155
Any pet under 6 months old	Y N	23 390	21 374	1.05	0.55, 2.02	0.998
Touch or handle other pets	Y N	45 342	67 313	0.61	0.40, 0.95	0.024

[2] Fisher's Exact Test
n.e not estimable

		Cases	Controls	Odds Ratio	95% CIs	p-value
Touch or handle any farm animals	Y N	70 344	43 348	1.65	1.07, 2.54	0.021
Touch or handle any zoo animals	Y N	4 395	4 380	0.96	0.20, 4.65	1.000[2]
Touch or handle any wild animals	Y N	12 384	8 375	1.46	0.55, 4.00	0.546
Touch or handle any manure or bird droppings	Y N	24 316	23 321	1.06	0.56, 2.01	0.967
Toileting contact with a child under 5 years of age	Y N	86 341	52 348	1.69	1.14, 2.51	0.008
Nappy changing contact with a child under 5 years of age	Y N	71 356	48 352	1.46	0.96, 2.22	0.073
Feeding contact with a child under 5 years of age	Y N	110 317	90 310	1.20	0.85, 1.67	0.311
Bathing or washing contact with a child under 5 years of age	Y N	111 316	95 305	1.13	0.81, 1.57	0.506
Provide personal care for adult or older child	Y N	36 374	32 354	1.06	0.63, 1.81	0.904
Contact with another person ill with diarrhoea	Y N	91 324	24 365	4.27	2.59, 7.10	<0.001
Did you drink unboiled tap water at home	Y N	347 67	342 54	0.82	0.54, 1.23	0.359
Number of glasses of tap water drunk a day	0 1 2 3 4 5 or more	67 54 57 65 47 73	54 65 74 88 57 33	 0.67 0.62 0.60 0.66 1.78	 0.39, 1.15 0.37, 1.05 0.36, 0.99 0.38, 1.16 1.00, 3.19	0.037[1]
Disruption to your water supply at home	Y N	27 321	25 353	1.19	0.65, 2.18	0.650
Any water discoloration	Y N	22 392	16 380	1.33	0.66, 2.72	0.490
Any altered taste to the water	Y N	14 400	4 392	3.43	1.03, 12.57	0.040
Any loss of water pressure	Y N	11 403	13 383	0.80	0.33, 1.95	0.751
Any other problems with water	Y N	17 397	11 385	1.50	0.65, 3.49	0.400
Did you drink unboiled tap water at somewhere other than home	Y N	212 153	193 162	1.16	0.85, 1.58	0.352
Did you use ice cubes	Y N	120 277	113 274	1.05	0.76, 1.45	0.813
Number of times ice cubes were used	0 1 2 3 4 5 or more	277 13 17 15 19 30	274 12 24 20 9 31	 1.07 0.70 0.74 2.09 0.96	 0.45, 2.56 0.35, 1.39 0.35, 1.55 0.88, 5.08 0.55, 1.68	0.944[1]

[1] Chi-Squared Test for Trend
[2] Fisher's Exact Test

		Cases	Controls	Odds Ratio	95% CIs	p-value
Drink any bottled water	Y N	164 228	148 232	1.13	0.83, 1.52	0.457
Number of glasses of bottled water a day	0 1 2 3 4 5 or more	228 31 34 12 16 21	232 49 37 12 9 8	 0.64 0.94 1.02 1.81 2.67	 0.38, 1.07 0.55, 1.59 0.42, 2.48 0.74, 4.53 1.10, 6.71	0.018[1]
Eat lettuce	Y N	161 212	186 183	0.75	0.55, 1.01	0.057
Eat other green salad	Y N	165 194	204 146	0.61	0.45, 0.83	0.001
Eat tomatoes	Y N	195 184	249 126	0.54	0.39, 0.73	<0.001
Eat coleslaw	Y N	83 267	116 233	0.62	0.44, 0.89	0.007
Eat raw vegetables	Y N	94 259	157 196	0.45	0.33, 0.63	<0.001
Eat fresh fruit	Y N	332 54	361 21	0.36	0.20, 0.63	<0.001
Eat rare steak	Y N	20 337	21 328	0.93	0.47, 1.83	0.940
Eat raw shellfish	Y N	17 337	14 334	1.20	0.55, 2.65	0.750
Eat uncooked soft cheese	Y N	85 270	116 238	0.65	0.46, 0.91	0.012
Eat uncooked hard cheese	Y N	243 125	281 85	0.59	0.42, 0.83	0.002
Eat yoghurt	Y N	288 90	292 78	0.85	0.59, 1.23	0.420
Eat ice cream	Y N	249 127	284 80	0.55	0.39, 0.78	<0.001
Eat cream	Y N	86 259	124 223	0.60	0.42, 0.84	0.003
Consume freshly pressed apple juice	Y N	41 311	59 291	0.65	0.41, 1.02	0.062
Eat barbecued meat	Y N	65 293	51 299	1.30	0.85, 1.99	0.235
Eat any new or unusual foods	Y N	43 330	20 359	2.34	1.30, 4.24	0.003
Drink pasteurised milk	Y N	306 98	322 73	0.71	0.49, 1.01	0.057
Eat cereal with pasteurised milk	Y N	302 103	316 79	0.73	0.52, 1.04	0.080
Drink unpasteurised milk	Y N	29 368	18 374	1.64	0.86, 3.15	0.144
Eat cereal with unpasteurised milk	Y N	28 370	19 375	1.49	0.79, 2.85	0.243
Regularly bite nails or chew fingers	Y N	157 258	134 261	1.19	0.88, 1.60	0.278

[1] Chi-Squared Test for Trend

		Cases	Controls	Odds Ratio	95% CIs	p-value
Smoke cigarettes or cigars	Y N	54 366	38 361	1.40	0.88, 2.24	0.162
Wash hands before eating or handling food	Always Usually Sometimes Never	103 207 99 10	102 187 102 5	 1.10 0.96 1.98	 0.78, 1.54 0.65, 1.42 0.64, 6.12	0.549
Your child ever eat soil	Y N	13 195	6 214	2.38	0.82, 7.23	0.125
Do you usually drink unboiled tap water at home	Y N	362 57	345 50	0.92	0.60, 1.42	0.768
Number of glasses of unboiled water usually drunk at home a day	0 1 2 3 4 5 or more	57 52 81 74 60 72	50 64 74 94 55 42	 0.71 0.96 0.69 0.96 1.50	 0.41, 1.25 0.57, 1.62 0.41, 1.16 0.55, 1.68 0.85, 2.67	0.061[1]
Use a water filter at home	Y N	39 373	34 360	1.11	0.66, 1.85	0.771
Do you usually drink unboiled tap water somewhere other than home	Y N	245 139	233 135	1.02	0.75, 1.39	0.950
Number of glasses of unboiled water a day usually drunk somewhere other than home	0 1 2 3 4 5 or more	139 77 66 35 15 13	135 82 68 30 12 11	 0.91 0.94 1.13 1.21 1.15	 0.61, 1.37 0.61, 1.46 0.64, 2.02 0.51, 2.88 0.46, 2.86	0.582[1]
If eat raw fruit and vegetables, are they normally washed before eating	Always Usually Sometimes Never	166 121 73 37	147 143 80 15	 0.75 0.81 2.18	 0.54, 1.04 0.55, 1.19 1.15, 4.14	0.005
Touch any equine animals	Y N	22 392	22 369	0.94	0.49, 1.81	0.968
Touch any sheep	Y N	18 396	10 381	1.73	0.74, 4.11	0.233
Touch any cattle	Y N	22 392	8 383	2.69	1.11, 6.70	0.024
Touch any fowl	Y N	5 409	9 382	0.52	0.15, 1.73	0.359
Touch any farm animals (other than equines, sheep, cattle or fowl)	Y N	7 407	2 389	3.35	0.63, 23.75	0.179

[1] Chi-Squared Test for Trend

		Cases	Controls	Odds Ratio	95% CIs	p-value
Health Authority	Bury and Rochdale	40	19			<0.001
	East Lancashire	14	46	0.14	0.06, 0.32	
	Liverpool	0	0	n.e.	n.e.	
	Manchester	40	37	0.51	0.25, 1.04	
	Morecambe Bay	8	4	0.95	0.25, 3.55	
	North West Lancashire	32	58	0.26	0.13, 0.53	
	North Cheshire	7	7	0.48	0.15, 5.55	
	Salford and Trafford	17	10	0.81	0.31, 2.09	
	Sefton	0	0	n.e.	n.e.	
	South Cheshire	42	51	0.39	0.20, 0.77	
	South Lancashire	1	1	0.48	0.83, 8.01	
	St Helens and Knowsley	5	4	0.59	0.14, 2.47	
	Stockport	43	29	0.70	0.34, 1.45	
	West Pennine	24	10	1.14	0.46, 2.85	
	Wigan and Bolton	27	23	0.56	0.26, 1.22	
	Wirral	15	6	1.19	0.40, 3.54	
	Bro Taf	1	0	n.e.	0.0004, ∞	
	Dyfed Powys	24	23	0.50	0.22, 1.09	
	Gwent	8	17	0.22	0.08, 0.61	
	Lechyd Morgannwg	5	10	0.24	0.07, 0.79	
	North Wales	74	45	0.78	0.40, 1.51	

An epidemiological study of sporadic cryptosporidiosis in Wales and North West Region: seroprevalence study

Supplementary report on the epidemiological study of sporadic cryptosporidiosis

Contents

Project participants

Principal Investigator for overall study:

Paul R Hunter, CDSC North West, Chester

Currently; Professor of Health Protection,
School of Medicine, Health Policy and Practice,
University of East Anglia
Norwich NR4 7TJ
Tel: 01603 591004
Email: paul.hunter@uea.ac.uk

Principal Investigator for seroepidemiolgy study:

Rachel M. Chalmers, PHLS Cryptosporidium Reference Unit, Swansea.

Project team:

Sara Hughes, CDSC Northwest, Chester.

Kristin Elwin, Stephen Hadfield, PHLS Cryptosporidium Reference Unit, Swansea.

Louise Hesketh, Preston Public Health Laboratory

Frank Lyons, Liverpool Public Health Laboratory

Summary

One approach to further investigate the differences in reported incidence of disease is to measure the extent of exposure to the organism in question in the population by testing for specific antibody responses. IgG responses to low molecular weight *Cryptosporidium* sporozoite antigens in adults have been shown to be consistent and of sufficient intensity to act as reliable markers of exposure. We investigated both seroprevalence and relative intensity of IgG antibody responses to the 15/17kDa *Cryptosporidium* sprorozoite antigen complex and 27kDa antigen using a Western blot procedure in sera from two towns in the North West of England, Liverpool and Preston. Although there are marked differences in the reported incidence of cryptosporidiosis between the areas studied, there was no significant difference between seroprevalence or relative intensity of antibody responses between the two areas. Similarly there was no significant difference in the rate of seroconversion. Seropositivity increased with age.

Background

Much of the published literature on the epidemiology of cryptosporidiosis is concerned with outbreaks of disease, particularly those caused by drinking mains water (Meinhardt *et al*., 1996; Smith and Rose, 1998). However, outbreaks represent only a small proportion of cases of infection, while the majority of infections are sporadic in that they are not linked to other known cases. The epidemiology of these sporadic cases is not fully understood, and it cannot be assumed that the causes of sporadic cryptosporidiosis are broadly the same as those for outbreaks or in roughly the same proportion. This assumption would grossly overestimate the contribution of mains water as a risk factor. Because outbreaks associated with drinking water tend to be larger than those associated with other causes, they are more easily identified. To date there have been very few studies of sporadic cryptosporidiosis. It would appear, however, that the incidence of cryptosporidiosis varies quite markedly from one region to another and from one health authority to another within the same region (Table1), suggesting that the epidemiology itself varies from one district to another.

One approach to further investigate the differences in reported incidence of disease is to measure the extent of exposure to the organism in question in the population by testing for specific antibody responses. IgG responses to low molecular weight *Cryptosporidium* sporozoite antigens in adults have been shown to be consistent and of sufficient intensity to act as reliable markers of exposure (Moss *et al*., 1998a; Moss *et al*., 1998b). The overall aim of this project is to investigate the epidemiology of sporadic cryptosporidiosis. This has been undertaken in two components:

- Seroprevalence study

  The seroprevalence study will ascertain the prevalence of exposure to *Cryptosporidium* in the community and enable the calculation of seroconversion rates, and is reported on here.

- Case Control study

The case control study has been undertaken to identify the main risk factors for sporadic cryptosporidiosis and elucidate further the role of mains drinking water. This has been reported on in “A case control study of sporadic cryptosporidiosis conducted in Wales and the North West region of England”, final report to DEFRA (Drinking Water Inspectorate) and United Utilities, 2003.

Table 1. **Annual incidence rate of cryptosporidiosis per 100,000 population for Health Authorities in North West region of England.**

	Year												
Health Authority	1990	1991	1992	1993	1994	1995	1996	1997	1998	1999	2000	2001	2002
Bury & Rochdale	19.6	18.5	29.3	25.8	14.9	27.2	17.7	23.6	16.4	22.6	27.9	11.9	9.6
East Lancashire	7.8	14.0	28.7	21.8	20.9	20.3	12.9	19.9	13.3	8.0	15.5	9.9	4.3
Liverpool	3.5	5.0	3.3	2.1	2.1	2.1	4.7	0.6	1.3	0.2	0	1.1	0.7
Manchester	25.1	42.4	43.9	29.4	18.3	6.2	28.3	28.6	14.2	15.1	43.5	12.0	13.7
Morecambe Bay	8.6	18.8	37.9	19.9	15.0	17.5	16.2	23.6	17.8	9.7	19.0	3.2	3.9
North Cheshire	1.5	7.0	10.0	8.4	3.2	1.9	4.9	0.4	3.2	1.5	5.8	4.8	2.9
NW Lancashire	34.2	34.5	56.1	37.2	50.6	62.7	24.0	62.5	42.6	56.1	64.1	8.0	9.3
Salford &Trafford	6.3	15.2	15.7	16.1	8.5	11.4	10.5	23.9	9.2	20.1	19.9	3.5	3.0
Sefton	2.7	4.1	5.1	4.4	7.2	4.1	3.1	7.2	2.1	2.4	3.8	1.4	3.2
South Cheshire	6.0	4.6	7.6	4.8	4.4	11.5	7.2	9.0	9.6	7.3	8.5	5.5	9.4
South Lancashire	1.0	0.6	6.5	11.3	2.3	4.5	6.1	9.1	15.8	45.6	42.5	13.1	14.7
St Helen's & Knowsley	0.0	1.1	0.0	0.2	0.7	0.0	0.2	2.0	2.0	0.4	2.4	1.5	2.1
Stockport	2.2	3.9	4.4	12.2	5.6	6.1	1.7	8.9	10.0	21.2	25.3	16.9	16.9
West Pennine	6.5	4.0	11.9	3.6	4.0	4.2	4.3	7.0	13.2	16.4	32.8	7.8	8.2
Wigan & Bolton	13.5	14.6	24.1	17.5	17.9	10.8	15.5	26.9	20.9	13.6	26.3	5.7	6.9
Wirral	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.9	1.2	0	0	0
North West Region	8.6	11.7	17.5	12.9	10.6	11.3	10.0	15.4	11.7	13.6	21.3	6.6	6.9

Introduction

The protozoan parasite *Cryptosporidium* is widely distributed, commonly occurring in the environment, and is a common cause of gastrointestinal disease in humans (Meinhardt *et al*., 1996). Multiple sources and routes of transmission contribute to a complex epidemiological picture, and little is known about the levels of endemic infection in the population. To ascertain the community prevalence of exposure to *Cryptosporidium* and enable the calculation of seroconversion rates, paired sera, collected at times separated by at least 4 months, were tested for IgG antibodies to the 15/17kDa *Cryptosporidium* sporozoite antigens and the 27kDa sporozoite antigen using a Western blot method. The Western blot has been previously shown to correlate better than enzyme linked immunosorbent assay to known risk factors for Cryptosporidium infection (Frost *et al*., 1998a). Of the 15/17kDa and 27kDa antigens, antibody responses to the former decline to baseline over 4-6 months post infection, representing a marker of recent infection while the latter remains elevated for some 6-12 months post infection providing a marker of slightly more distant infection in terms of time (Frost *et al*., 2002).

In this study, sera were collected from Liverpool and Preston Public Health Laboratories (PHLs), locations selected because Liverpool has low reported incidence rates for cryptosporidiosis whereas Preston covers areas with high reported rates (North West, East and South Lancashire) (Table 1). This provides an opportunity to compare reported disease incidence rates with observed seroprevalence and give an estimate of the overall disease burden. Although antibodies to these antigens appear to be a reliable marker of exposure to *Cryptosporidium* in adults, antibody responses in children a less well characterised and it is not clear that the antigens normally used in adults are appropriate for younger age groups (Robert Morris, Tuffts University, personal communication).

Seroprevalence Study Methods

Up to 500 randomly selected, anonymised paired serum samples, taken from adult patients (≥15 years old) at an interval of at least 4 months were collected from each of the two PHLS laboratories in Preston and Liverpool. The sera were left over from those submitted by local general practitioners (GPs) and local hospital trusts for a variety of clinical reasons unrelated to *Cryptosporidium* infection and were tested with the permission of PHLS Ethics Committee and Local Research Ethical Committee approval. Caldicott principles of patient confidentiality were adhered to throughout the study, and PHLS laboratories conform to all PHLS policies on patient confidentiality. All research is audited through the PHLS Research and Development Assessment Review panels.

The sera were analysed for *Cryptosporidium* sporozoite antibodies at the PHLS Cryptosporidium Reference Unit, Swansea using a Western blot method as previously described (Frost *et al*., 2000). Briefly, a sporozoite antigen preparation (supplied by T. Muller, Lovelace Respiratory Research Institute, Albuquerque, USA) was separated into component antigen proteins by sodium dodecyl sulphate-polyacrylamide mini-gel electrophoresis. The proteins were then transferred by semi-dry transfer onto nitrocellulose sheets, which were then placed into a multi-screen apparatus that allows isolation of vertical strips of the blot for contact with either test or control sera. Positive control serum was also supplied by T. Muller (Lovelace Respiratory Research Institute, Albuquerque, USA). Test and control sera were prepared as a 1/50 dilution in PBS/0.3% $Tween_{20}$. Bound human antibodies in the sera were detected by incubation with a secondary biotinylated mouse anti-human IgG antibody. The bound secondary antibody was then detected by reaction with streptavidin alkaline phosphatase, which was visualised by a colour reagent containing 5-bromo-4-chloro-3-indolyl phosphate as substrate and nitro-blue tetrazolium as chromagen (Frost *et al*, 1998). Intensitometric data were obtained on the serological responses to three sporozoite antigens: 15 and 17kDa antigens which, since mini-gels do not resolve the antigens separately, are here referred to as the 15/17kDa antigen complex (Frost *et al*., 1998) and the 27KDa antigen, using a digital camera and KDS1D analysis software (Kodak). The relative intensity of the

antibody response was calculated as a percentage of that in the positive control on each gel.

A range of cut off values was explored to define positivity, and thus estimate seroprevalence, based on the intensity of the band(s) of interest, at ≥10%, 20% and 30% of that of the relevant antibody in the positive control on each gel. Given that a positive response has not been defined in the literature and that further data are awaited on time series analyses from patients following microbiologically defined cryptosporidiosis, advanced analyses were based upon distribution of relative intensity rather than defined cut off values for positivity. Seroconversion was explored based upon a change in relative intensity >10% when first and second sera were compared. Age distribution of antibody responses was based upon 10 year age groups.

Data analysis was done with SPSS or StatsDirect (Buchan 2000).

Results

A total of 248 suitable pairs of sera were collected from Preston PHL between July 2000 and September 2002. Of the paired sera, 57 (23%) were from men, 188 (76%) were from women and for 3 (1%) the gender was not known. The age range at the time of the first specimen was 15 to 89 years (mean = 36, median = 32 years). The mean time difference between the collection of the first and second serum samples was 344 days (range 109 to 750 days, median = 318 days).

A total of 84 suitable pairs and 152 single sera were collected from Liverpool PHL between July 1995 and July 2000. Of the paired sera, 27 (32%) were from men, 55 (66%) were from women and for 2 (2%) the gender was unknown. The age range at the time of the first specimen was 17 to 59 years (mean = 33, median = 31 years). The mean time difference between the collection of the first and second serum samples was 557 days (range 182 to 1356 days, median = 473 days). Of the single sera 45 (30%) were from men

and 107 (70%) were from women. The age range was 19 to 73 years (mean =32, median = 30 years).

Differences between Preston and Liverpool paired sera in terms of age of the donor at the time of the first sample were not significant (Mann-Whitney two sample test Z=-1.853, P=0.064) and neither were the gender differences significantly different (Uncorrected Chi^2 = 3.425, P = 0.064). For most analyses, the single sera from Liverpool were grouped with the first samples of the paired sera from Liverpool.

The seroprevalence of the 15/17kDa antigen in the first sera from Preston ranged from 15% to 27% and in the first or single sera from Liverpool ranged from 13% to 31%, and of the 27kDa antigen in Preston from 11% to 30% and in Liverpool from 11% to 33%, depending on the chosen cut off to define positivity (Table 2). The overall seroprevalence, at a 10% cut off value, for the 15/17kDa antigen was 141/484 (29%), which was not significantly different to 154/484 (32%) for that of the 27kDa antigen (Uncorrected Chi^2 = 0.824, P = 0.364). Indeed, no significant differences were detected at any of the chosen cut off values.

No significant differences were observed in seroprevalence in the first samples between the two locations using any of the three possible cutoff values for either the 15/17kDa or 27kDa antigens (Table 2).

Table 2. Seroprevalence of the 15/17kDa and 27kDa *Cryptosporidium* sporozoite antigens in first or only sera from Liverpool and first sera from in Preston

	Positive response to 15/17kDa antigen			Positive response to 27kDa antigen		
Cut off* for positivity	Preston (n=248)	Liverpool (n=236)	Chi square; p; df=1	Preston (n=248)	Liverpool (n=236)	Chi square; p; df=1
10%	67 (27%)	74 (31%)	1.10, 0.294	75 (30%)	79 (33%)	0.58, 0.446
20%	52 (21%)	47 (20%)	0.08, 0.774	45 (18%)	36 (15%)	0.72, 0.395
30%	37 (15%)	30 (13%)	0.49, 0.483	27 (11%)	25 (11%)	0.01, 0.917

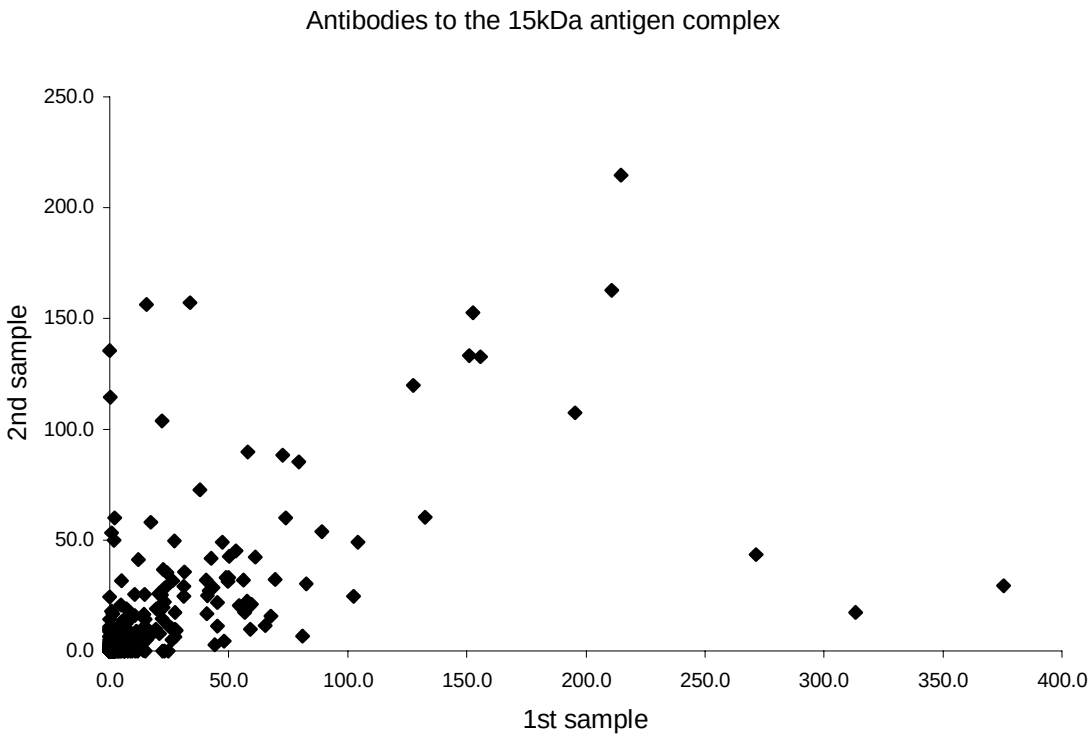
*defined as relative intensity of test sera compared with positive control

Seroconversion was observed in 27 (8%) sera measured at the 15/17kDa antigen response, 31 (9%) sera measured at the 27kDa and 49 (15%) by either antigen. Although, the rate of seroconversions was higher in the Liverpool sera, this was due to the longer time between sample dates in the Liverpool sera compared to the Preston sera. Table 3 gives the conversion rates per 100 person years and the significance of any difference between the rates. The overall conversion rate was 13.54 (95%CIs 10.00-17.89) per 100 person years. It can be seen that the seroconversion rates did not differ significantly between the two locations.

The intensity of antibody responses relative to control sera, to the 15KDa antigen between 1st and 2nd samples is correlated (Wicoxon signed ranks test, z = -2.465, p=0.014) (Figure 1). The relative intensity of the antibody responses to the 27KD antigen between 1st and 2nd samples is very highly correlated (Wicoxon signed ranks test, z = -3.688, p=<0.001) (Figure 1).

Table 3. Seroconversion rates in paired sera from Liverpool and Preston

	Liverpool (n=84)	Preston (n=248)	Chi^2	P
No. serum pairs	84	248		
Total person-days between samples	46,756	85,391		
15/17 KDa Ag marker				
No. seroconversions	11	16		
Rate/ 100 person years	8.58	6.83	0.3391	0.5603
(95% CIs)	(4.27-15.37)	(3.91-11.10)		
27 KDa Ag marker				
No. seroconversions	16	15		
Rate/ 100 person years	12.48	6.42	3.5721	0.0588
(95% CIs)	(7.15-20.29)	(3.58-10.59)		
Either Ag marker				
No. seroconversions	21	28		
Rate/ 100 person years	16.39	11.97	1.1976	0.2738
(95% CIs)	(10.15-25.08)	(7.96-17.30)		



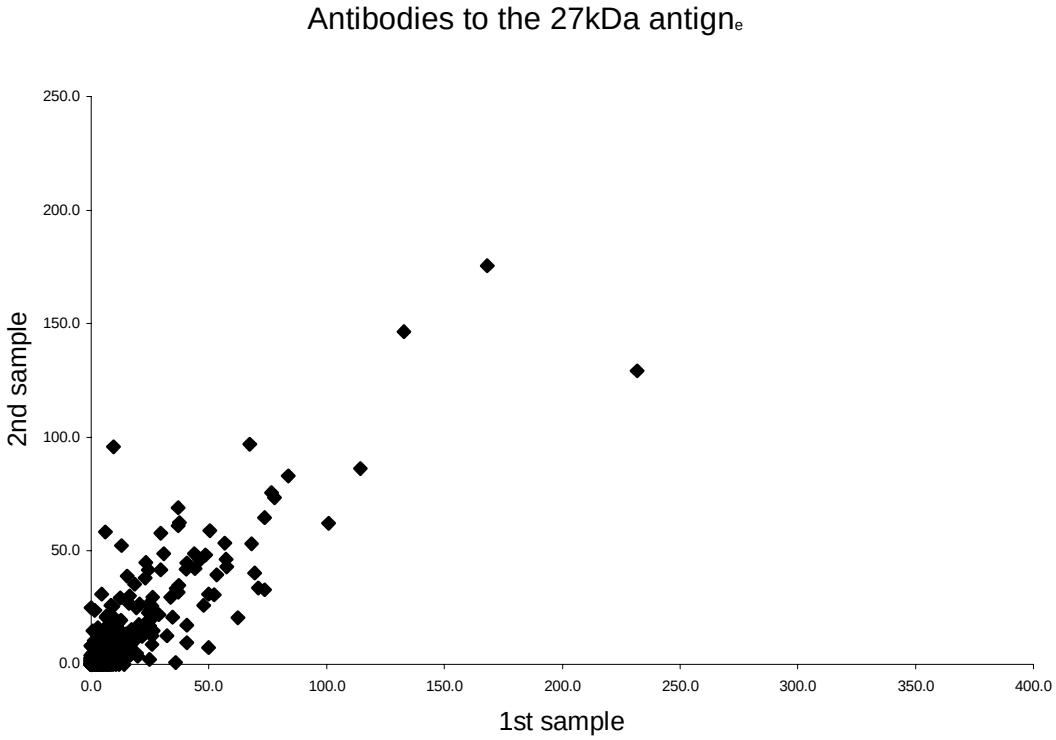


Figure 1. Relative intensity of antibody responses to 15/17kDa antigen complex and 27kDa antigen in sera from the North West of England

There was a strong trend to increased positivity with age seropositivity for both the 15/17kDa antigen complex and the 27kDa, though this was most marked for the anti - 15/17 KDa antigen (Table 4).

Table 4. Proportion of sera positive in relation to age, using the 10% cutoff

Age group	Total in age group	15/17 KDa Ag		27 KDa Ag	
		Positive	% Positive	Positive	% Positive
15 to 29	197	38	19.3	50	25.4
30 to 49	230	76	33.0	82	35.7
50 to 89	57	27	47.4	22	38.6
Chi^2 for trend		20.13		5.972	
P value		<0.0001		0.0145	

Discussion

Cryptosporidium infection elicits an antibody response in most exposed individuals, and the Western blot method is regarded as providing a reliable measure of their presence in sera from adult populations (Moss *et al*., 1998a; Moss *et al*., 1998b; Frost *et al*., 1998a). Responses to the 15/17kDa and 27 kDa sporozoite antigens appear to peak some 4 to 6 weeks after infection and while the 15/17kDa marker declines to baseline over 4 to 6 months, the 27kDa remains elevated for 6 to 12 months (Frost *et al*., 2002). In a study of paired sera taken 6 months and then 2 years following a drinking water outbreak of cryptosporidiosis (Frost *et al*., 1998b), the + 6 month sera showed a relatively weak response to the 15/17kDa antigen, probably having already declined in that time to baseline, while the mean response to the 27kDa antigen was strong. Two years later, the mean response to the 15/17kDa antigen remained at baseline, while that of the 27kDa had declined to a mean intensity of 54% of that measured at 6 months.

Based on prior knowledge of antibody responses, it might therefore be expected that the prevalence of antibodies to the 27kDa antigen would be higher in randomly selected population sera than the 15/17kDa antigen complex. In this study, however, there was no clear difference in the prevalence of antibodies to these two antigens. Other studies have been published regarding seroprevalence in population-derived samples, and while some have shown differences in prevalence between these antibody responses, others have not (Table 5). There is currently no consensus for the definition of a positive antibody response to define *Cryptosporidium* seropositivity in the Western blot. In their study of blood donors in Jackson County following an outbreak of cryptosporidiosis, Frost and colleagues (1998a) chose a cut off value of relative intensity of 35% of the positive control citing evidence from paired sera collected over an unspecified period of time that individuals may maintain responses of up to 30% for extended periods while responses >35% declined. The seroprevalence was 22%, 26% and 48% for the 15kDa, 17kDa and 27kDa antigens respectively. Using the same cut off value, seroprevalence of 26% was detected for the 15/17kDa antigen complex and 39% for the 27kDa antigen in gay and bisexual men (Caputo *et al*., 1999). By contrast, in some studies blots have been assessed

by eye for a detectable antibody response (Moss *et al*., 1994; Moss *et al*., 1998b; Isaac-Renton *et al*., 1999). Similarly in one study Frost and colleagues (2000a) used a detectable response (defined from quantitative anaylsis as >5% positive control) as the cut off value for positivity and in another study Frost and colleagues (2000b) used a detectable response defined as 1% or more. We found that analysing blots by eye equated to between 5% and 10% relative intensity depending on the intensity of the positive control. However, until further work has been undertaken to define criteria for positive sera, focussing on changes in mean relative intensity of antibody responses is perhaps more useful for analysis of data and generation of information regarding population exposure to *Cryptosporidium*. Moss and colleagues (1998b) explored changes in reactivity using intensitometry, and found that increases in reactivity were more likely in experimentally infected volunteers developing cryptosporidiosis than in those who were asymptomatically infected or oocyst-negative, and variation in mean net intensity has been correlated with cases / non cases in that symptomatic infection was associated with consistent changes in antibody responses (Moss et al., 1998a). Thus relative intensity is a useful measure for monitoring exposure to *Cryptosporidium* at the population level.

Table 5. Prior seroprevalence data on IgG responses to the 15/17kDa antigen complex and 27kDa *Cryptosporidium* sporozoite antigens

Study group or population	Assay and definition of positivity	Seroprevalence 15/17kDa antigen	Seroprevalence 27kDa antigen	Reference
Blood donors in Jackson county 4-6 months following the end of a drinking water-associated outbreak	Western blot 35% relative intensity cut off	83/380 (22%) 15kDa 97/380 (26%) 17kDa	182/380 (48%)	Frost et al., 1998a
Non-outbreak (ie. not reporting foreign travel and not known to have been exposed) banked serum samples from CDC employees	Western blot by eye	46/74 (62%)	68/74 (92%)	Priest et al., 1999
1987 Carrolltown, Georgia outbreak		Early outbreak 11/33 (33%) Late outbreak 91/96 (95%)	17/33 (52%) 95/96 (99%)	
1994 Walla Walla County, Washington outbreak. Known to have been exposed; sera taken 6 weeks after peak in epidemic		28/35 (80%)	34/35 (97%)	
3 communities in Canada: Deep wells, no oocysts detected	Western blot By eye	49/283 (17%)	45/283 (16%)	Isaac-Renton et al., 1999
Surface water from a protected watershed, intermittently containing oocysts		549/1442 (38%)	223/1442 (16%)	
Surface water frequent detection of Crypto		81/219 (37%)	34/219 (16%)	
Gay and bisexual male volunteers in a cohort study in Australia	Western blot 35% relative intensity cut off	61/236 (26%)	92/236 (39%)	Caputo et al., 1999

Left over sera from routine tests Collingwood residents, Ontario, Canada, following an outbreak Toronto residents as comparison for Collingwood	Western blot >5% relative intensity cut off	61/89 (69%) 36/80 (45%)	78/89 (88%) 36/80 (45%)	Frost et al., 2000a
Sydney blood donors following the water crisis Melbourne blood donors for comparison	Western blot 1% relative intensity cut off	59/104 (57%) 64/104 (61%)	69/104 (66%) 81/104 (78%)	Frost et al., 2000b
Two city study, blood donors Surface water city Groundwater city	Western blot Range of cut off values explored eg. given at 10%	189/462 (41%) RR1.69	221/462 (48%) RR=1.35	Frost et al., 2002

Given the differences in reported incidence of cryptosporidiosis between Preston (high) and Liverpool (low), it is interesting that there was no difference in seroprevalence to either the 15/17kDa antigen or the 27kDa antigen indicating similar rates of exposure to *Cryptosporidium*.

The rate of seroconversion in this study is surprisingly high. Given the short lived nature of the antibody response, especially to the 15/17kDa Ag (discussed above) and the prolonged time span between many first and second sera in this study, the estimated seroconversion rate is likely to be an underestimate.

During the period of collection of the Liverpool sera the mean annual number of reports was 1.36 per 100,000 population and for Preston this was 23.43 per 100,000 population. So in Liverpool for every case reported to CDSC there were an estimated 12,051 seroconversion and for Preston there were 511 seroconversions. It would appear that whilst infection with *Cryptosporidium* is very common, few infections lead to symptomatic infections. The rate of acute gastroenteritis, from any cause, in the community is only 19.4 episodes per 100 person years (Wheeler *et al*., 1999), little more than the mean seroconversion rate of 13.54 found in this study. There is still the issue of why case ascertainment in Preston is some 20 times greater than in Liverpool. Given that reporting mechanisms are supposed to be similar in the two locations, this finding is a cause of some concern (Chalmers *et al*., 2002).

In our study we found a gradual increase in the strength of antibody response up to the age of 60. It is likely that multiple exposure throughout life may elicit a greater response and that seroprevalence studies may underestimate extent of exposure to single infections. Certainly increased antibody responses has been found to be significantly greater in symptomatic volunteers than other volunteers (Moss et al., 1998b). Volunteer studies also suggest that IgG antibody responses reflect protective immunity to illness following infection with Cryptosporidium oocysts (Moss et al., 1998b). If prior infection is protective, does this place antibody-negative people at greater risk of clinical infection?

While population-based serological studies are useful in examining exposure to *Cryptosporidium* further data is required from on-going studies to better characterise intensity and lifespan of serological responses.

Conclusions

1. There is no significant difference between sero-positivity of sera from Liverpool or Preston or in the rate of seroconversions between the two areas. This is despite a very large difference in the number of reports of diagnosed infections in the two health authorities.

2. Response to the 27KD Ag is fairly consistent, whereas that to 15KD Ag is less so.

3. There is a gradual increase in seropositivity with age.

References

Buchan, I.E. (2000) StatsDirect Statistical Software version 1.605. http://www.statsdirect.com

Caputo, C., Forbes, A., Frost, F., Sinclair, M.I., Kunde, T.R., Hoy, J.F. and Fairley, C.K. (1999) Determinants of antibodies to *Cryptosporidium* infection among gay and bisexual men with HIV infection. *Epidemiology and Infection* **122**, 291-297

Chalmers, R.M., Hughes, S., Thomas, A., Woodhouse, S., Thomas, P.D. and Hunter, P. (2002) Laboratory ascertainment of Cryptosporidium and local authority public health policies for the investigation of sporadic cases of cryptosporidiosis in two regions of the United Kingdom. *Communicable Disease and Public Health* **5**: 114-118.

Frost, F.J., de la Cruz, A.A., Moss, D.M., Curry, M. and Calderon, R.L. (1998a) Comparisons of ELISA and Western blot assays for detection of *Cryptosporidium* antibody. *Epidemiology and Infection* **121**, 205-211

Frost, F.J., Calderon, R.L., Muller, T.B., Curry, M., Rodman, J.S., Moss, D.M. and de la Cruz, A.A. (1998b) A two-year follow-up survey of antibody to *Cryptosporidium* in Jackson County, Oregon following an outbreak of waterborne disease. *Epidemiology and Infection* **121**, 213-217

Frost, F.J., Muller, T., Craun, G.F., Fraser, D., Thompson, D., Notenboom, R. and Calderon, R.L.(2000a) Serological analysis of a cryptosporidiosis epidemic. *International Journal of Epidemiology* **29**, 376-379

Frost, F.J., Muller, T.B., Fairley, C.K., Hurley, J.S., Craun, G.F. and Calderon, R.L. (2000b) Serological evaluation of *Cryptosporidium* oocyst findings in the water supply for Sydney, Australia. *International Journal of Environmental Health Research* **10**, 35-40

Frost, F.J., Muller, T., Craun, G.F., Lockwood, W.B. and Calderon, R.L. (2002) Serological evidence of endemic waterborne *Cryptosporidium* infections. *Annals of Epidemiology* **12**, 222-227

Isaac-Renton, J., Blatherwick, J., Bowie, W.R., Fyfe, M., Khan, M., Li, A., King, A., McLean, M., Medd, L., Moorehead, W., Ong, C.S. and Robertson W. (1999) Epidemic and endemic seroprevalence of antibodies to *Cryptosporidium* and *Giardia* in residents of three communities with difference drinking water supplies. *American Journal of Tropical Medicine and Hygiene* **60**, 578-583

Meinhardt, P.L., Casemore, D.P. and Miller, K.B. (1996) Epidemiological aspects of human cryptosporidiosis and the role of waterborne transmission. *Epidemiologic Reviews* **18**, 118-136

Moss, D.M., Bennett, S.N., Arrowood, M.J. et al., (1994) Kinetic and isotypic analysis of specific immunoglobulins for crew members with cryptosporidiosis on a U.S. Coast Guard cutter. *Journal of Eukaryotic Microbiology* **41**, 52s-77s

Moss, D.M., Bennett, S.N., Arrowoods, M.J., Wahlquist, S.P. and Lammie, P.J. (1998a) Enzyme-linked immunoelectronictransfer blot analysis of a cryptosporidiosis outbreak on a United States coast guard cutter. *American Journal of Tropical Medicine and Hygiene* **58**, 110-118

Moss, D.M., Chappel, C.L., Okhuysen, P.C., DuPont, H.L. and Arrowood, M.J. (1998b) Hightower AW, Lammie PJ.The antibody response to 27- 17- and 15-kDa *Cryptosporidium* antigens following experimental infection in humans. *Journal of Infectious Diseases* **178**, 827-833

Priest, J.W., Kwon, J.P., Moss, D.M., Roberts, J.M., Arrowood, M.J., Dworkin, M.S., Juranek, D.D. and Lammie, P.J. (1999) Detection by enzyme immunoassay of serum immunoglobulin G antibodies that recognise specific *Cryptosporidium parvum* antigens. *Journal of Clinical Microbiology* **37**, 1385-1392

Smith, H.V. and Rose, J.B. (1998) Waterborne cryptosporidiosis: current status. *Parasitology Today* **14**, 14-22

Wheeler, J.G., Sethi, D., Cowden, J.M., Wall, P.G., Rodrigues, L.C., Tompkins, D.S., Hudson, M.J. and Roderick, P.J. (1999) Study of infectious intestinal disease in England: rates in the community, presenting to general practice, and reported to national surveillance. *British Medical Journal* **318**, 1046-1050.

Enhanced surveillance in Wales and the North West Region of England

Supplementary report on the epidemiological study of sporadic cryptosporidiosis

Contents

Project participants

Principal Investigator:

Paul R Hunter, CDSC North West, Chester

Currently; Professor of Health Protection,
School of Medicine, Health Policy and Practice,
University of East Anglia
Norwich NR4 7TJ
Tel: 01603 591004
Email: paul.hunter@uea.ac.uk

Project team:

Sara Hughes, Qutub Syed & Sarah Woodhouse, CDSC Northwest, Chester.

Rachel Chalmers, PHLS Cryptosporidium Reference Unit, Swansea.

Ian Lake, School of Environmental Sciences, University of East Anglia.

Summary

This report supplements the report of the steering group investigating the epidemiology of sporadic cryptosporidiosis in the North West and Wales. The enhanced surveillance part of the project was primarily designed to identify cases for inclusion in the case-control study, though as a secondary function we aimed to include a geographical analysis of the distribution of cases.

Some 747 reports of cases were made to CDSC North West of which 10 were excluded, as they were duplicate reports. A further 88 reports were excluded as they had incorrect or incomplete postcodes, leaving 649 reports for analysis. Cases were plotted on the maps of water supply zone and water quality area boundaries, provided by the two main water utilities (United Utilities and Welsh Water).

It was notable that there were major spatial variations in attack rate across the North West and Wales. The most dramatic example was the large difference between the Greater Manchester conurbation with many reports and the Liverpool with none. There is no obvious explanation for this difference. An analysis of the distribution of cases in the Greater Manchester area showed no correlation with any of five water supplies that serve the conurbation.

Introduction

This short report describes the results of the enhanced surveillance study set up as part of the case-control study of sporadic cryptosporidiosis.

The purpose of the enhanced surveillance was to ensure a timelier and more complete dataset for cryptosporidium cases than could have been achieved from laboratory reporting alone. Laboratories in the North West of England and Wales routinely send reports of cryptosporidium cases to the Communicable Disease Surveillance Centre (CDSC). However information via this route is often slow, with some laboratories only reporting to CDSC every few weeks or longer. In addition, the laboratory reports that are received contain minimal and varied information, most only giving the case's sex, date of birth and date of specimen.

A quicker, alternative route was achieved with the co-operation of Consultants in Communicable Disease Control (CCDC's) based at each of the 21 Health Authorities in the North West of England and Wales. CCDC's also routinely receive notifications of cryptosporidium cases from the laboratories. Notifications are received promptly, and include additional information such as name and address of case. This additional information allows more detailed localisation of cases to enable geographical mapping.

Methods

CCDC's were approached via the Communicable Disease Task Force meeting in the North West of England and the Consultants in Communicable Disease Control meeting in Wales. They were asked to forward details of cryptosporidium cases to CDSC upon notification from the laboratory. A data collection form was completed for each case, giving the following details: name, address, postcode, date of birth, GP name, GP address and date of notification. The form was faxed or e-mailed to CDSC North West as soon as possible.

Enhanced surveillance for the North West of England and Wales were set up separately, North West England in mid December 2000 and Wales in February 2001. Both ran until February 2002.

To check for accuracy, the data were audited every 2 months. Each CCDC was sent a list of the cases they had notified to CDSC North West in the preceding 2 months. Any cases that had not been notified were forwarded to CDSC. The flow of information from a cryptosporidium case to CDSC North West is shown in figure 1.

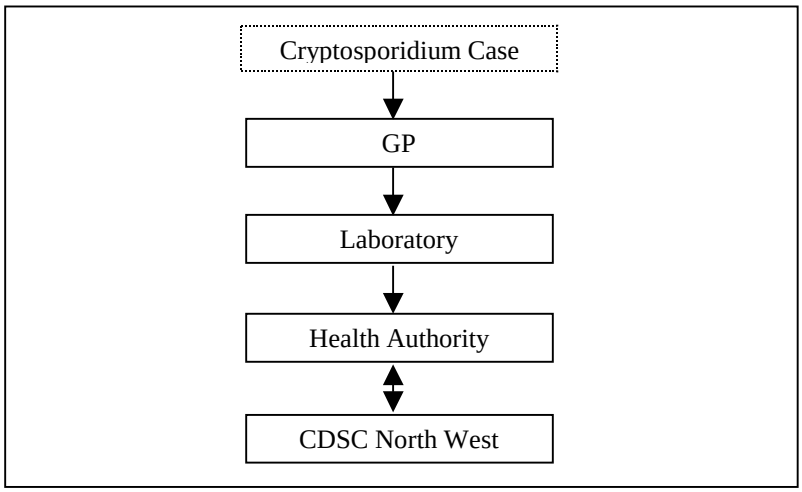


Figure 1. *Flow of information for Enhanced Surveillance.*

The main objective of the enhanced surveillance project was the timely identification of cases for inclusion in the case-control study. However, postcodes were used for geographical analysis in order to identify whether there was any spatial relationship with particular water supply zones.

The first stage in the geographical analysis was to check the 747 records for possible duplicate records. These were selected on the criteria of 2 individuals with identical names, dates of birth and postcodes being present in the database. Given that a postcode contains on average only 15 addresses the chances of these being legitimate is highly unlikely. Through this procedure 10 records were deleted from the database. Consequently 737 cases of cryptosporidiosis were identified during the period of enhanced surveillance.

The next step was to assign a grid reference to each postcode and this was achieved using the Royal Mail Postcode Address File. Eighty eight records were excluded as either, an incomplete postcode was entered into the cryptosporidiosis database or a match could not be found in the postcode address file. Therefore, in total the database was reduced to 649 cryptosporidiosis cases. These were plotted as points against a backdrop of the water supply zones for the two main water utilities. The water supply zone and water quality area boundaries were provided by the two main water utilities (United Utilities and Welsh Water).

Using the GIS each case was also assigned its corresponding water supply zone and the number of cases in each WSZ was divided by the population, based upon data supplied by the two water utilities, to produce the attack rate maps. The analysis was undertaken in ArcGIS 8.1 using point in polygon techniques (Burrough & McDonnell 1998).

Results

Table 1 shows the number of records from each health authority and the number of exclusions, including reasons for exclusion.

Table 1. Health authority of reported cases, including reasons for exclusion from analysis

Health Authority	Total records	Included in analysis	Excluded	Reasons for excluding post codes				% excluded
				Incorrect	Duplicate	Incomplete	Missing	
Bro Taf	2		2	2				100
Bury and Rochdale	51	43	8	4	2	1	1	15.7
Dyfed Powys	49	44	5	5				10.2
East Lancashire	47	45	2	2				4.3
Gwent	12	12	0					0
Iechyd Morgannwg	6		6	6				100
Morecambe Bay	13	10	3			3		23.1
Manchester	69	48	21	3		10	8	30.4
N Cheshire	6	6	0					0
North Wales	121	111	10	9		1		8.3
North West Lancashire	74	59	15	3	2		10	20.3
South Cheshire	63	63	0					0
South Lancashire	20	20	0					0
Salford	34	28	6			4	2	17.6
St Helens	8	8	0					0
Stockport	66	60	6	1	1		4	9.1
West Pennine	32	29	3		1	1	1	9.4
Wigan and Bolton	46	35	11	2	4	4	1	23.9
Wirral	28	28	0					0
TOTAL	747	649	98	37	10	24	27	13.1

The results of the geographical analyses are shown in figures 2 to 7. Figures 2 and 3 show the geographical distribution of individual cases by indicating a dot on the map of the water supply zones (water quality area for Wales). Figures 4 and 5 indicate the attack rates for each zone/area where the shading indicates a range of attack rates. Care should be taken in interpreting the zone rates as the populations covered by each zone/area varied substantially. In some zones high attack rates were seen despite only a single case being identified because of a low denominator population.

It can be seen that there is substantial spatial variation in the distribution of reported cases. In part, this variation can be explained variation in population density. However, much of the variation is unexplained. For example, reports from Liverpool are very uncommon, whilst reports from Greater Manchester are very common.

It was decided to investigate the excess case reporting from Greater Manchester in further detail to look for any possible association with water supplies. Water to the Greater Manchester area comes from five main water treatment works; Lostock (derived from Thirlmere in the Lake District and chlorinated but not filtered), Woodgate Hill (derived from Haweswater and Windermere via the Watchgate Treatment Works near Kendal where the water is treated by rapid gravity sand filtration, though not chemically coagulated before spring 2003), Arnfield-Godley (chemical coagulation, clarification and rapid gravity sand filtration), Buckton Castle (chemical coagulation, dissolved air flotation and rapid gravity sand filtration) and Wybersley (chemical coagulation, dissolved air flotation and rapid gravity sand filtration).

In order to determine whether there was any relationship between attack rate and water supply, all water supply zones in the North West that received any water from one or more of these five supplies were identified. Figure 6 shows the approximate distribution of water from these five treatment works. The shaded areas indicate the dominant water source to each zone. However, there is a substantial degree of mixing and many zones receive water from more than one treatment works. Also many zones received water from these five work, but do not receive a dominant supply from one. For each of these water supply zones, the proportion of the supply from each treatment works were obtained from United Utilities. The correlation between the attack rate and proportion of water from each treatment works was tested using Kendall's rank correlation (table 2). The figure adjusted for ties was used. There was no significant correlation between water source and attack rate.

Table 2. Correlation between water supply zone specific attack rate and proportion of water received from each of the five main water treatment works supplying Greater Manchester.

Water treatment works	Z	P value
Lostock	-1.084	0.2782
Woodgate Hill	1.713	0.0867
Arnfield – Godley	-1.186	0.2353
Buckton Castle	-0.628	0.5294
Wybersley	0.451	0.6517

Figure 2

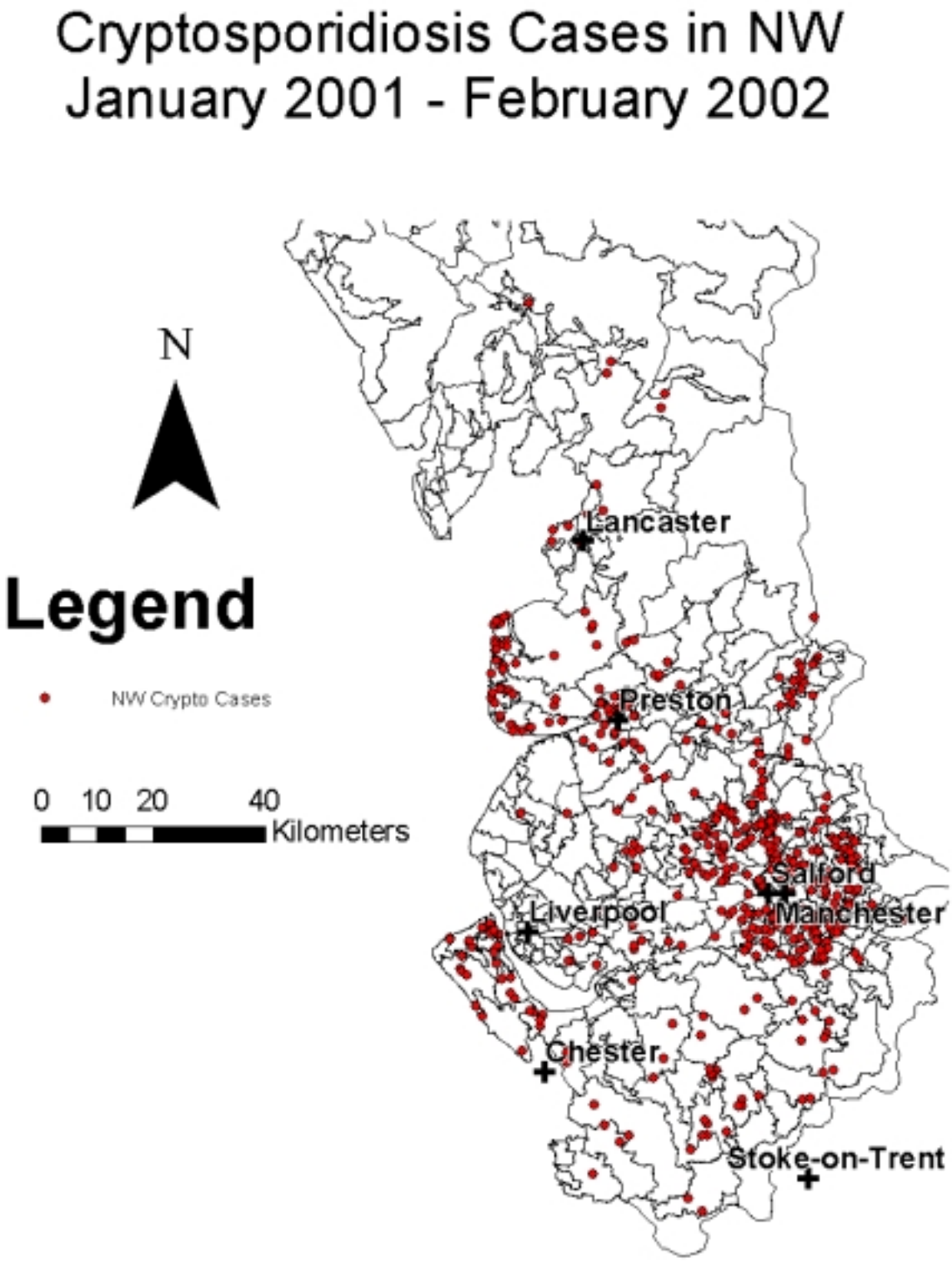

Cryptosporidiosis Cases in NW
January 2001 - February 2002
N
Legend
NW Crypto Cases
0 10 20 40
Kilometers
Lancaster
Preston
Salford
Manchester
Liverpool
Chester
Stoke-on-Trent

Figure 3

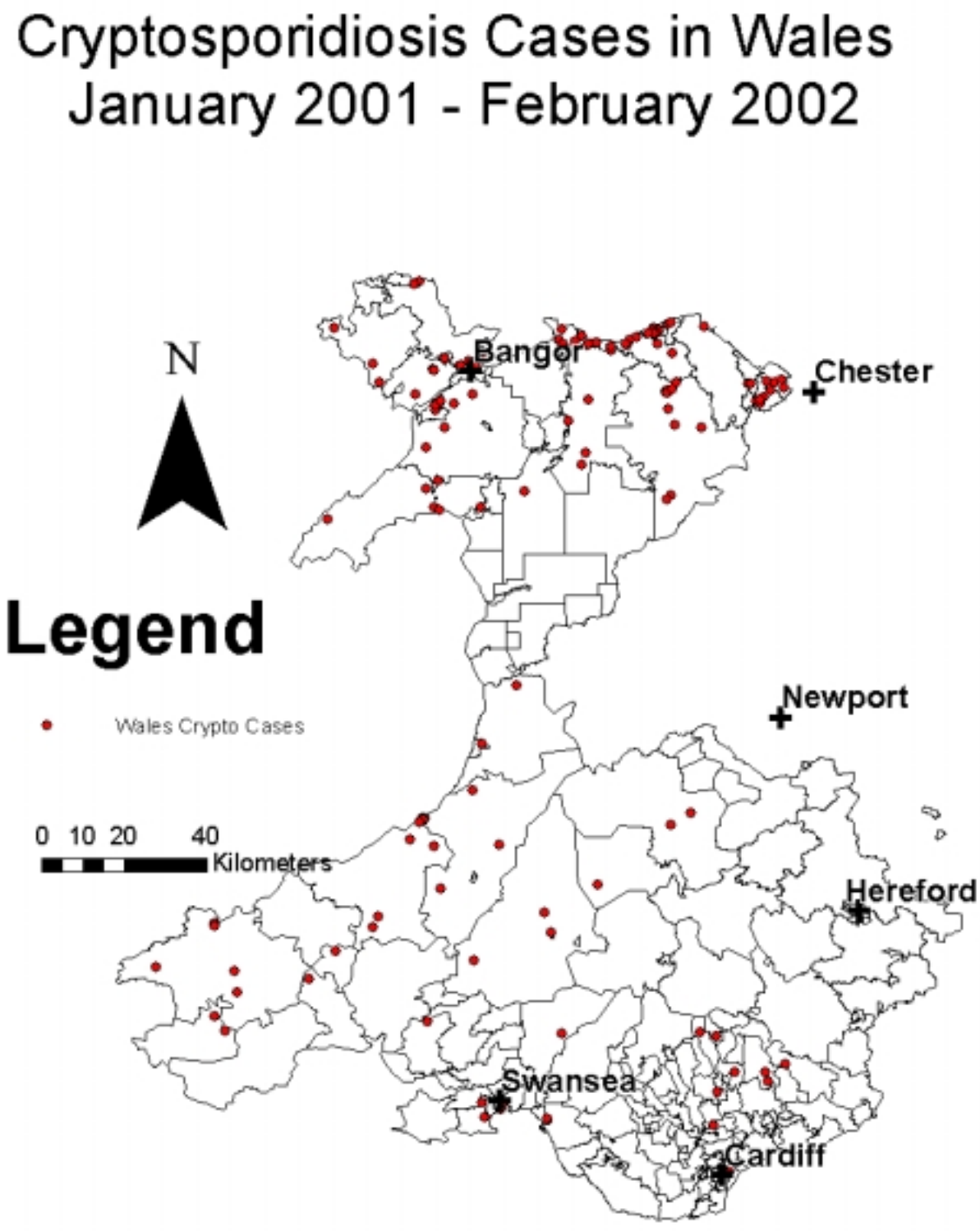

Cryptosporidiosis Cases in Wales
January 2001 - February 2002
N
Legend
Wales Crypto Cases
0 10 20 40
Kilometers
Bangor
Chester
Newport
Hereford
Swansea
Cardiff

Figure 4

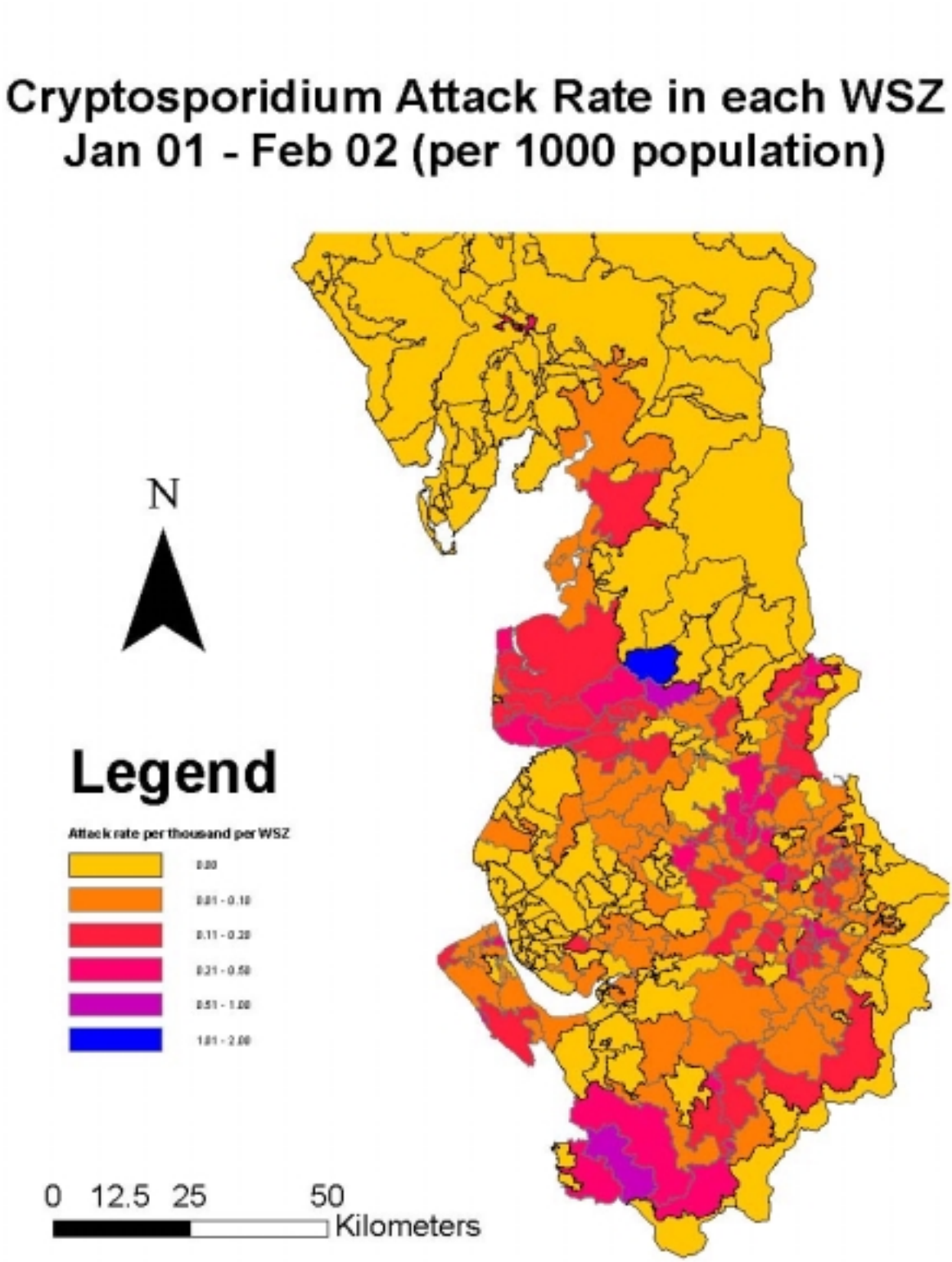

Cryptosporidium Attack Rate in each WSZ
Jan 01 - Feb 02 (per 1000 population)
N
Legend
Attack rate per thousand per WSZ
0.00
0.01 - 0.10
0.11 - 0.20
0.21 - 0.50
0.51 - 1.00
1.01 - 2.00
0 12.5 25 50
Kilometers

Figure 5

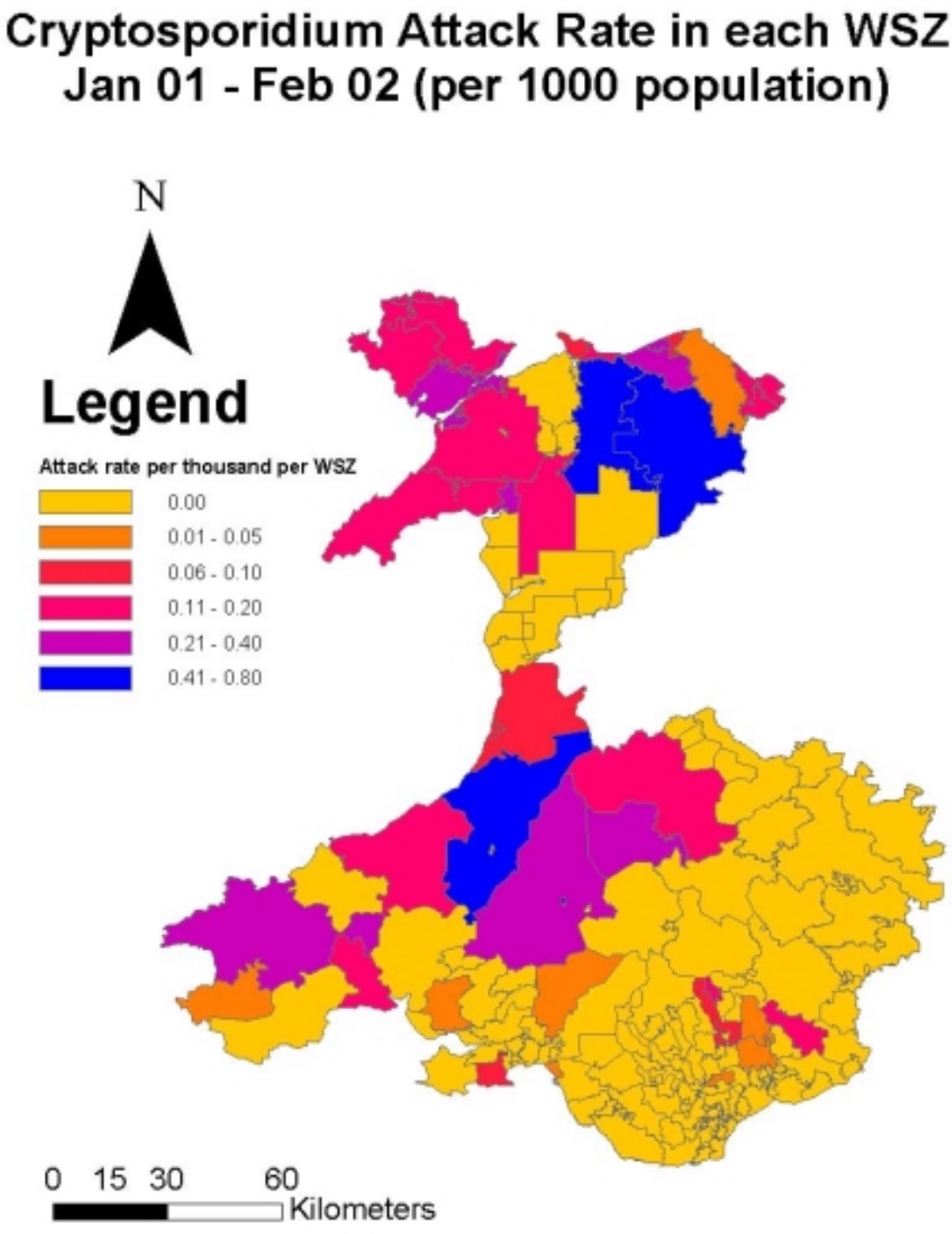
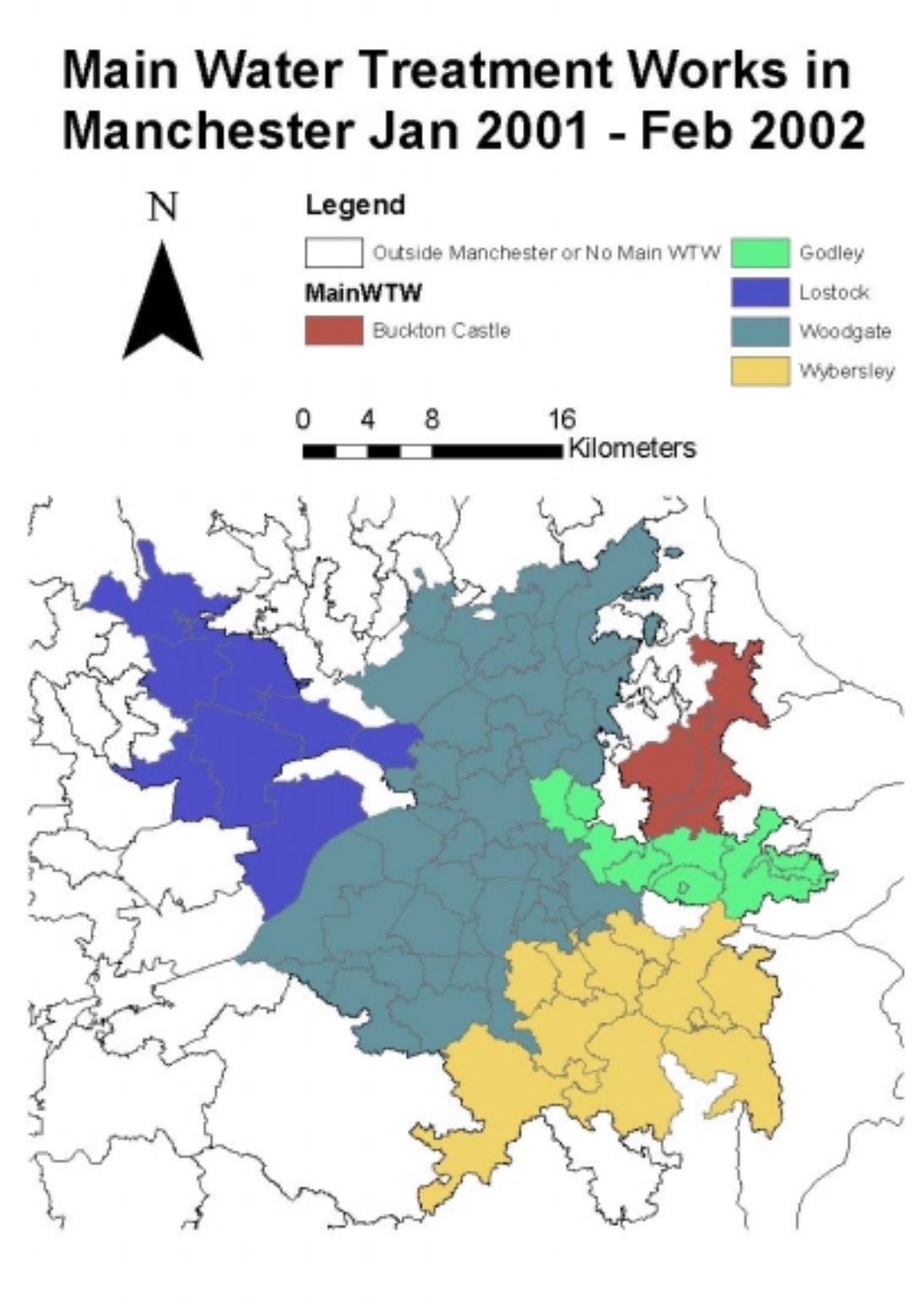

Cryptosporidium Attack Rate in each WSZ
Jan 01 - Feb 02 (per 1000 population)
N
Legend
Attack rate per thousand per WSZ
0.00
0.01 - 0.05
0.06 - 0.10
0.11 - 0.20
0.21 - 0.40
0.41 - 0.80
0 15 30 60
Kilometers

Figure 6


Main Water Treatment Works in Manchester Jan 2001 - Feb 2002
N
Legend
Outside Manchester or No Main WTW
MainWTW
Buckton Castle
Godley
Lostock
Woodgate
Wybersley
0 4 8 16
Kilometers

Discussion

As already mentioned, care should be taken in the interpretation of this analysis. It is notable that the proportion of reports that could not be allocated a correct postcode varied from one health authority to another to some extent. Also variation in attack rate between water supply zones or water quality areas was as likely to be due to differences in population size as to differences in reported cases. This was most obvious in zones/areas with relatively small population sizes where random effects could have a particularly important affect. However, there are a number of obvious features.

The most obvious is the large number of cases from the Greater Manchester conurbation. This covered the Bury and Rochdale, Manchester, Salford, Stockport, West Pennine, and Wigan and Bolton Health Authorities. This excess of cases in Manchester is even more remarkable when compared with the virtual absence of cases from the Liverpool conurbation (Liverpool, Sefton and St Helen's Health Authorities). The reason for the excess of cases in Greater Manchester is unclear. Although different reporting habits could play a part, we doubt that it could explain more than a small part of the difference. Reporting practices are not that greatly different across the North West (Chalmers et al. 2002).

An alternate explanation could be that the increase represents different water supplies. Salford, and Wigan and Bolton Health Authorities get much of their water supply from Thirlmere, a supply known to be prone to contamination by *Cryptosporidium* (Hunter et al. 2001), none of the others have been implicated in outbreaks of disease. However, it would appear that the attack rates did not vary in any consistent way in relation to water source and so a waterborne hypothesis for this excess could not be proven. Analysis was restricted to Greater Manchester as analysis of all reports in the North West could be subject to confounding as a result of geographical variation in reporting behaviour, whereas the Health Authorities in Greater Manchester share a very similar notification system. .

A further explanation could be that the Manchester population experience other risk factors more commonly than the Liverpool population. If people from Manchester were

more likely to come into contact with farm animals or travel abroad more frequently than people from Liverpool, this could explain the difference. Unfortunately we do not have sufficient data from the case control study to resolve this question. The sero-epidemiology study, currently underway, may be able to determine whether the low reporting rate from Liverpool is real or not. Furthermore, it will be interesting to see whether the completion of an adequate water filtration plant for the Thirlmere supply has much, if any, impact the number of reports.

In addition to Greater Manchester, there are also areas of increased reporting from North Wales and from North West Lancashire. These hotspots also remain unexplained. North West Lancashire, however, receives much of its water from Thirlmere and a water source cannot be excluded. However, many cases were reported from the Fylde peninsular which only receives a small proportion of its water from Thirlmere.

In conclusion the use of GIS to study the spatial distribution of cases has been useful in identifying differences in the distribution of cases, but not necessarily for identifying the reasons for those differences. We agree with Dangendorf *et al.* (2002) that GIS will contribute substantially to our understanding of the contribution of drinking water to human disease as it aids the identification of possible associations between disease and particular water supplies, provided sufficient information is collected to enable accurate location of cases.

References

Burrough, P. A. and McDonnell, R. A. (1998). Principles of Geographical Information Systems. Oxford, Oxford University Press.

Chalmers, R.M., Hughes, S., Thomas, A., Woodhouse, S., Thomas, P.D. and Hunter, P. (2002) Laboratory ascertainment of Cryptosporidium and local authority public health policies for the investigation of sporadic cases of cryptosporidiosis in two regions of the United Kingdom. *Communicable Disease and Public Health* **5**, 114-118.

Dangendorf, F., Herbst, S., Exner, M. and Kistemann, T. (2002). Geographical Information Systems. In: Hunter PR, Waite M, Ronchi E (eds). *Drinking Water and Infectious Disease: Establishing the Links*. CRC Press, Bocata Raton, 2002, pp 143-153.

Hunter, P.R., Syed, Q. and Naumova, E.N. (2001) Possible undetected outbreaks of cryptosporidiosis in areas of the North West of England supplied by an unfiltered surface water source. *Communicable Disease and Public Health* **4**, 136-138.