Cryptosporidiosis:

A report on the surveillance and epidemiology of *Cryptosporidium* infection in England and Wales

Gordon Nichols, Rachel Chalmers, Iain Lake, Will Sopwith, Martyn Regan, Paul Hunter, Pippa Grenfell, Flo Harrison, Chris Lane

Drinking Water Directorate Contract Number DWI 70/2/201.

1 Summary

Disease

1.1 Cryptosporidiosis is a diarrhoeal disease that is commonest in young children, can infect people of all ages and is severe in people with an immune deficiency.

1.2 Surveillance has shown a predominantly spring and autumn distribution of cases over the period 1989 to 2000.

1.3 There has been an overall reduction in cryptosporidiosis in the first half of the year since 2001, but no similar reduction has occurred in the autumn.

1.4 The evidence suggests that the decrease in the first half of the year is related to improved drinking water quality, particularly in the North West where substantial new water treatment, including filtration has been installed to treat supplies that were previously unfiltered.

1.5 National infectious disease surveillance provides further evidence, on top of investigations of *Cryptosporidium* outbreaks and analytical studies of sporadic cryptosporidiosis, that some drinking water supplies have been responsible for a substantial burden of this diarrhoeal disease over the last few years.

1.6 Changes in human infectious disease surveillance indicate that the 1999 *Cryptosporidium* regulations have had a significant beneficial impact on waterborne disease in England and Wales. However, there may still be a burden of disease associated with drinking water and the epidemiological evidence is far from clear about the remaining causes of cases.

1.7 The routes by which of *Cryptosporidium* cases in the second half of the year are infected is unclear, although swimming and travel both appear to play an important part in the epidemiology of cryptosporidiosis in this part of the year.

1.8 There is evidence that swimming pools within England and Wales are contributing to an increase in cryptosporidiosis within local communities in the autumn period. There have been regular examples of people falling ill on holiday and, this may account for the autumn incidence of cases and the increased risk of spread of disease.

1.9 While travel related cryptosporidiosis has been recognised for years, two large outbreaks in the UK in different years (2000, 2003) associated with hotel pools in Majorca appear to have caused cases across England and Wales. This highlights the potential for sporadic cases across England and Wales to be related to a common holiday destination either within England and Wales or further afield.

1.10 Outbreaks related to drinking water have declined in recent years but there are still outbreaks linked to swimming pools and farm visits. Good up-to-date advice on how to conduct an outbreak investigation associated with these main routes of infection is desirable.

Water

1.11 Monitoring data from at risk sites has shown that a number of water supplies occasionally contain oocysts. The significance of these has always been questioned because the oocysts may be non-viable and may be of a species that is not commonly found in human cases. It remains possible that low counts of oocysts represent some risk of infection.

Laboratory methods

1.12 Evidence indicates that the routine laboratory staining methods currently used for screening human faecal samples for *Cryptosporidium* oocysts may be missing about a half of all the cases. In addition a number of hospital laboratories adopt restrictive selection criteria for testing faecal samples with the results that further cases may not be diagnosed.

1.13 The genotyping of isolates of *Cryptosporidium* to species level has provided clear information on the changing distribution of the two main species (*C. parvum* and *C. hominis*) within the human population. It has been useful in identifying species-specific risk factors. There is a strong case for adopting the strategy of typing all isolates in future.

1.14 Sub-typing *Cryptosporidium* oocysts at a level below species level has generated much new information and has the potential to provide new insights into the transmission of disease.

Surveillance

1.15 The improvements in the timeliness and completeness of reporting to national surveillance have increased the ability to detect national increases in cases in a timely manner. This improvement needs to continue.

1.16 The increase in the capture of the post-code of patients in a way that does not compromise patient confidentiality allows the ability to conduct geographic analytical studies that have not previously been possible. This should allow clearer investigations into the relationships between the risk status of water supplies and human disease.

2 Introduction

2.1 Cryptosporidiosis is an important cause of diarrhoeal disease. Surveillance has been conducted by the Communicable Diseases Surveillance Centre (CDSC) which was part of the Public Health Laboratory Service (PHLS) and subsequently the Centre for Infections (CfI) which is part of the Health Protection Agency (HPA) since 1983 and there have been 149 outbreaks that have been attributed to various sources / vehicles; public drinking water supplies, private drinking water supplies, swimming pools, other recreational water, animal contact, petting farms, food borne, person-to-person and other sources. The occurrence of *Cryptosporidium* outbreaks linked to water supplies led to specific *Cryptosporidium* Regulations in 1999 and health surveillance data has demonstrated these have had a beneficial impact on the occurrence of illness. The extent to which water supplies continue to pose an occasional risk to health remains unclear, but other sources of contamination remain a cause for concern.

2.2 The Drinking Water Inspectorate commissioned this report to summarise and disemminate updated information on *Cryptosporidium* infections within England and Wales.

2.3 The work has been completed by Gordon Nichols, Pippa Grenfell and Chris Lane from the HPA Centre for Infections, Rachel Chalmers from the *Cryptosporidium* Reference Unit, Iain Lake, Flo Harrison and Paul Hunter from the University of East Anglia and Will Sopwith and Martyn Regan from the Health Protection Agency North West. Additional proof reading assistance was provided by Corin Amar and Jim McLauchlin of the HPA Centre for Infections.

2.4 The work has been funded by the Water Directorate of Defra through the Drinking Water Directorate under the Contract Number DWI 70/2/201.

3 *Cryptosporidium* biology and life cycle

3.1 *Cryptosporidium* species live inside the epithelial cells of enterocytes within the intestinal tract of a variety of vertebrates, but some species can infect the respiratory tract.

3.2 A growing number of species have been shown to cause human disease although *C. parvum* (previously *C. parvum* Genotype 2) and *C. hominis* (previously *C. parvum* Genotype 1) remain the main species encountered in England and Wales.

3.3 The life cycle, oocyst details, typing, population genetics and genomes of *Cryptosporidium* are outlined in Appendix 1.

4 Surveillance of *Cryptosporidium*

4.1 Routine epidemiological surveillance for cryptosporidiosis allows the detection of changes in the temporal, geographic and age distribution of disease and can identify outbreaks of infection, or changes in the pattern of sporadic cases. In addition, routine specialised laboratory surveillance can generate information on changes in the type and pathogenicity of the parasite. Enhanced surveillance is used to investigate the sources of outbreaks and gathers more specific and detailed data on likely risk factors. Such investigations often result in communication of credible risks to industry (including those responsible for drinking water, swimming pools, farms, tourism etc.) and can define the requirements for more detailed research studies. The principles of surveillance are outlined in Appendix 2.

4.2 Infrastructures for surveillance of *Cryptosporidium*.

4.3 The Health Protection Agency (HPA) came into being in April 2003 and inherited a range of local operational services derived from those previously situated in the old Health Authorities, Local Councils and the Public Health Laboratory Service. These were often set up using the principle of “fit for purpose” and a variety of different surveillance systems persist across England and Wales.

4.4 All health regions contribute laboratory reports of cryptosporidiosis to the national Health Protection Agency database through CoSurv (NHS regional boundaries match those of regional government offices). There were 104 Health Authorities (set up in 1974) and these changed to 28 (plus Wales) Strategic Health Authorities (in 2002) and these have now been reduced to 10 (plus Wales). This electronic reporting system is linked directly to local NHS Trust laboratory systems. Reporting of all positively diagnosed specimens of *Cryptosporidium* electronically through the regional office is encouraged but not mandatory. The quality of associated demographic data received from laboratories varies in completeness and timeliness.

4.5 Some regions routinely operate enhanced surveillance for cryptosporidiosis where each laboratory diagnosed case is also reported to a local Health Protection Unit and followed up with a patient interview/questionnaire to gather more detailed information on potential risk factors. Enhanced surveillance is often maintained in regions where there has been a particularly high incidence of disease or where there is a particular public health interest.

4.6 Enhanced surveillance data is also often collected at a Local Authority level by Environmental Health Officers. This may be undertaken routinely on all cases or in response to a particular incident and may not be reported to a national system.

4.7 A proportion of laboratory isolates are also submitted to the UK *Cryptosporidium* Reference Unit for typing purposes. There is not a uniform detailed enhanced surveillance system maintained throughout England and Wales, and this obviously has implications for retrospective analysis of nationwide risk factors.

4.8 Within Wales, the National Public Health Service (NPHS) provides the Infection and Communicable Disease Service (ICDS) that incorporates the microbiology laboratories and Communicable Disease Surveillance Centre (which previously comprised the PHLS in Wales) and the Health Protection Teams (formerly employed by the Health Authorities). NPHS provides a range of operational and strategic information services that embrace both hospital and community practice. The UK *Cryptosporidium* Reference Unit is located in Swansea and is managed by NPHS (see Appendix 3)

4.9 The national resources within the Colindale site have changed, with the Communicable Disease Surveillance Centre (CDSC) and the Specialist and Reference Microbiology Services (SRMD) merging to form the Centre for Infections (CfI) and within this a merging of the Gastrointestinal Diseases Department and the Environmental Surveillance Unit with the Laboratory of Enteric Pathogens and the Food Safety Microbiology Laboratory under the new Department of Gastrointestinal Diseases (Appendix 4). This centre is where much of the development work on *Cryptosporidium* methods has been undertaken in the past, including monoclonal antibodies, PCR typing, national surveillance and epidemiology.

4.10 The HPA Gastrointestinal Diseases Programme Co-ordinators work with the Regional Food Water and Environmental (FWE) Co-ordinators that are currently in place to form the hub of a local gastrointestinal disease network to inform and underpin the Health Protection Agency Gastrointestinal Diseases Programme. These networks may also be fora for sharing surveillance information on cryptosporidiosis and for initiating action within Local and Regional Services (Appendix 5).

4.11 A Network of GI & FWE Specialists provides support to GI and Food and Water Scientists involved in both service and R&D work. The Network aims to develop contacts for research and potential collaborations, identify gaps in the GI/FWE knowledge base and identify research needs (Appendix 6).

4.12 The level of service provided by the UK *Cryptosporidium* Reference Laboratory is:

- Identification by microscopy 2 working days
- Typing by PCR 5 to 10 working days (depending upon complexity of the work involved and current workload)
- During outbreak investigations, 24 hour turnaround time is often achieved.

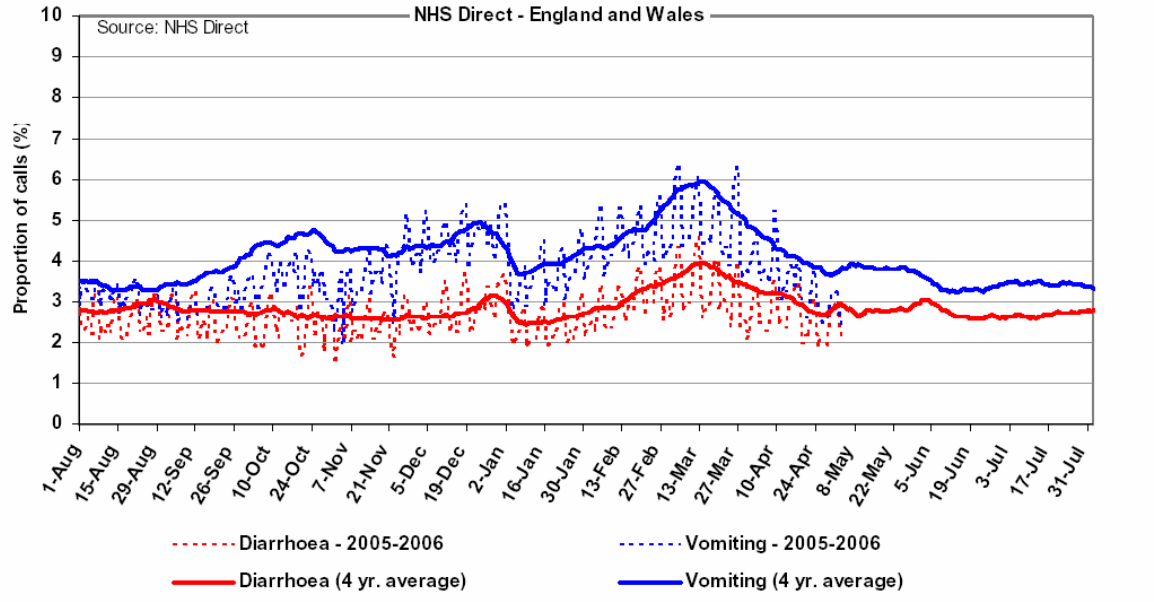
Where a cluster or outbreak is identified, reports of typing information are provided to the relevant Health Protection Unit on an incident-by-incident basis.

4.13 Systems for data transmission between *Cryptosporidium* Reference Unit and National *Cryptosporidium* surveillance at CfI are not in place for England and Wales and need to be established. For Scotland, a service level agreement is in place to fund the typing of all isolates and data are reported from the UK *Cryptosporidium* Reference Unit to Health Protection Scotland through the ECOSS system via NHSNet. The systems for Northern Ireland are more or less the same as those for England and Wales.

4.14 Surveillance using patient symptoms through NHS Direct (Syndromic surveillance)

4.15 Syndromic surveillance is that based on people's reported symptoms rather than laboratory diagnosed disease. A national UK surveillance system currently uses data from a health helpline (NHS Direct) in an attempt to provide early warning of a bio-terrorist attack, or an outbreak caused by a more common infection (Figure 1). This system can provide useful information on gastrointestinal infections.

Figure 1. Example of syndromic surveillance based on NHS Direct (Syndromic Surveillance Bulletin 234) GASTROINTESTINAL CALLS: Daily 'diarrhoea' and 'vomiting' calls as a proportion of total calls (2005-2006), compared with 4-year averages.



4.16 To determine whether syndromic surveillance would be useful in identifying outbreaks of *Cryptosporidium* a study was carried out involving superimposing data from a historical outbreak of cryptosporidiosis (Willocks et al., 1998) onto a statistical

model of NHS Direct call data using calls about diarrhoea (a proxy for cryptosporidiosis) (Cooper et al., 2006). It was concluded that the NHS Direct surveillance system is currently unlikely to detect an event similar to the cryptosporidiosis outbreak. It predicted that there would be only a 4% chance of detecting an outbreak if one-twentieth of cryptosporidiosis cases telephoned the helpline.

4.17 Surveillance of people with diarrhoeal symptoms can be conducted through general practitioners, and there are three systems of surveillance using general practitioners data (Appendix 7).

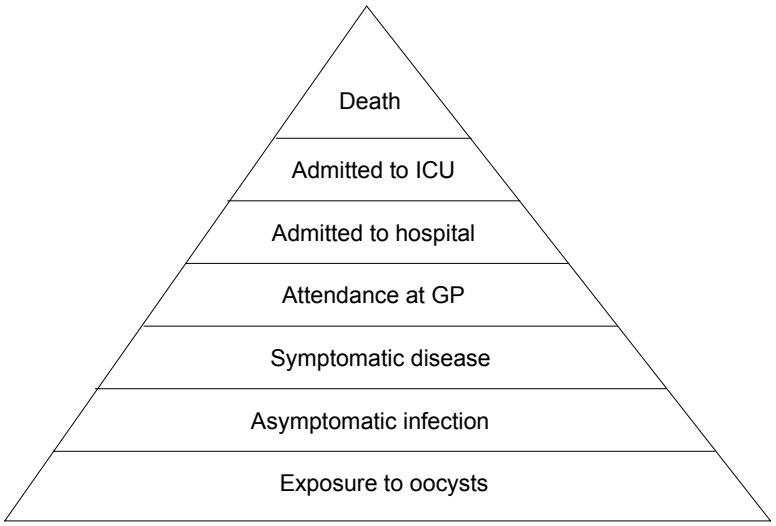
5 Factors that influence *Cryptosporidium* surveillance

5.1 A number of factors influence the conclusions that can be drawn from analysis of national surveillance data. These include behavioural, social, technical, and institutional matters all of which mean that the data will not necessarily be an exact reflection of disease within the population.

5.2 Spectrum of disease

5.3 There is a spectrum of disease resulting from exposure to *Cryptosporidium* oocysts that can be simplified into a pyramid (Figure 2). People are probably exposed to *Cryptosporidium* oocysts throughout their life and this exposure will not result in infection in all cases, particularly where the dose is small and the species involved is not *C. hominis* or *C. parvum*. When infection occurs it may not result in overt disease, particularly if the person has had a previous *Cryptosporidium* infection. When symptomatic disease does occur most of the affected people will seek medical advice, usually from their GP, but for the very young, the immunocompromised or the elderly, the symptoms can be severe enough to result in admission to hospital. In cases where there is severe dehydration, or an underlying predisposing disease such as HIV, infection may be very severe and lead to death. Although the severity and importance of opportunistic infections including cryptosporidiosis has been reduced since the introduction of highly active anti-retroviral therapy (HAART) (Hommer et al., 2003; Nannini and Okhuysen, 2002; Ives et al., 2001; Manfredi, 2000) many people are not diagnosed for HIV infection until they present with AIDS defining symptoms.

Figure 2. The pyramid of disease severity



5.4 The surveillance pyramid

5.5 Surveillance detects only a percentage of disease occurring in the population within a community and most infections are not diagnosed (Figure 3). There is under reporting at most levels depicted in Figure 3. If a person becomes ill with diarrhoea he/she will not necessarily immediately attend a general practitioner. Some patients rarely attend a GP whereas others commonly do. There can be a delay between the disease onset and attendance at a GP surgery because the person cannot or will not attend. For *Cryptosporidium*, as for other enteric pathogens, analysis of the dates of specimens recorded through national surveillance shows that fewer specimens are collected at week-ends and this is presumed to reflect lack of ready weekend access to general practitioner and laboratory services in some parts of the country (Figure 4).

5.6 The decisions taken by GPs on whether to take a faecal sample from someone with diarrhoea are based on clinical need and practice policies. If a faecal sample is not taken there will be no microbiological analysis and therefore nothing reported through laboratory surveillance. Sometimes faecal samples are requested by the Environmental Health Practicioners investigating the source of an outbreak in the community or in families. Similarly some physicians request specimens in relation to patients admitted to hospital.

Figure 3. The surveillance pyramid

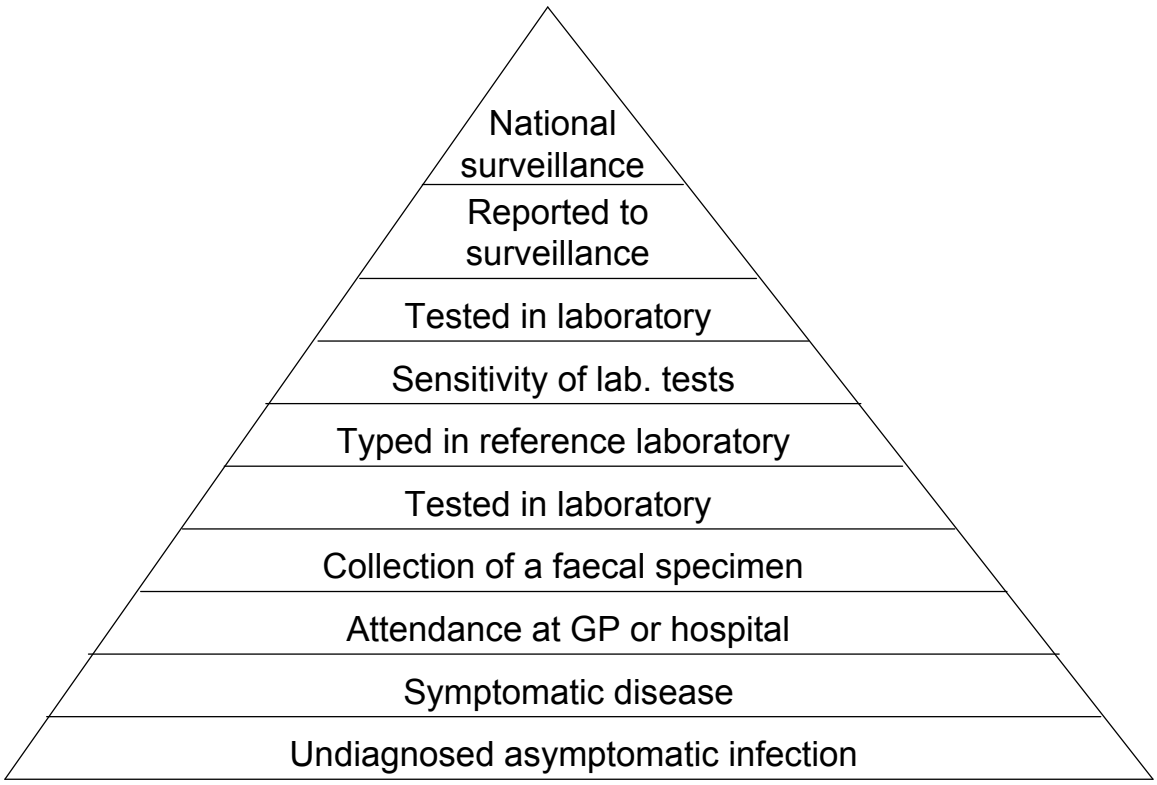
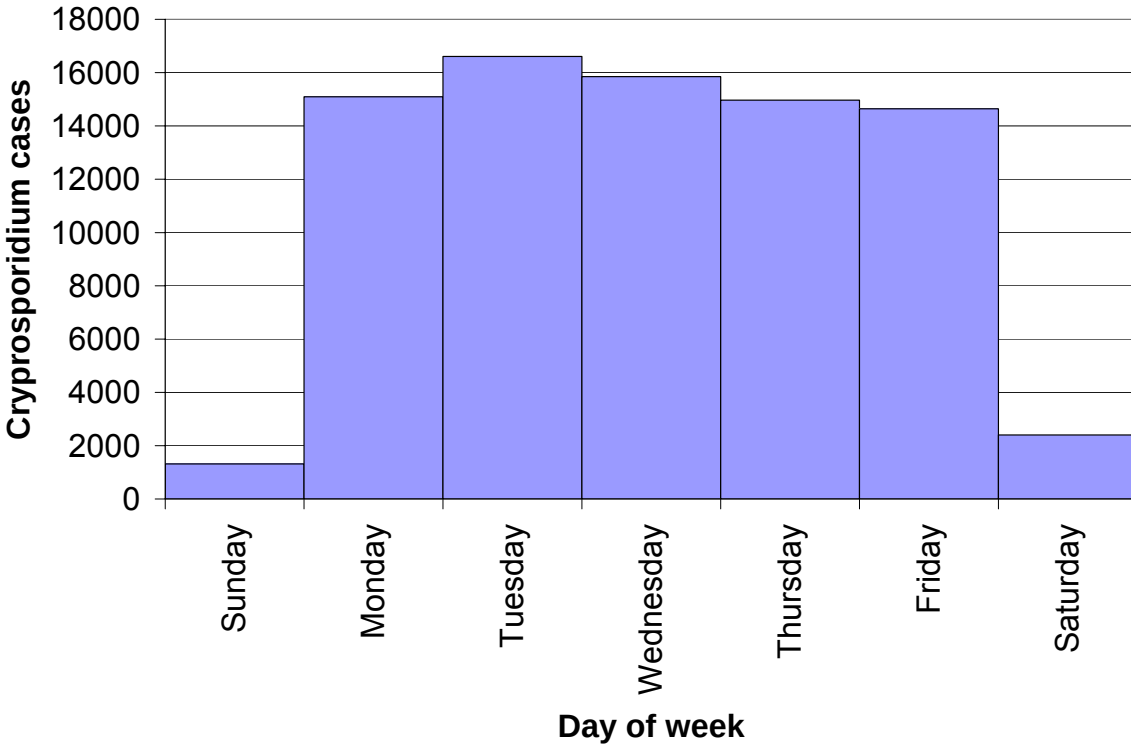


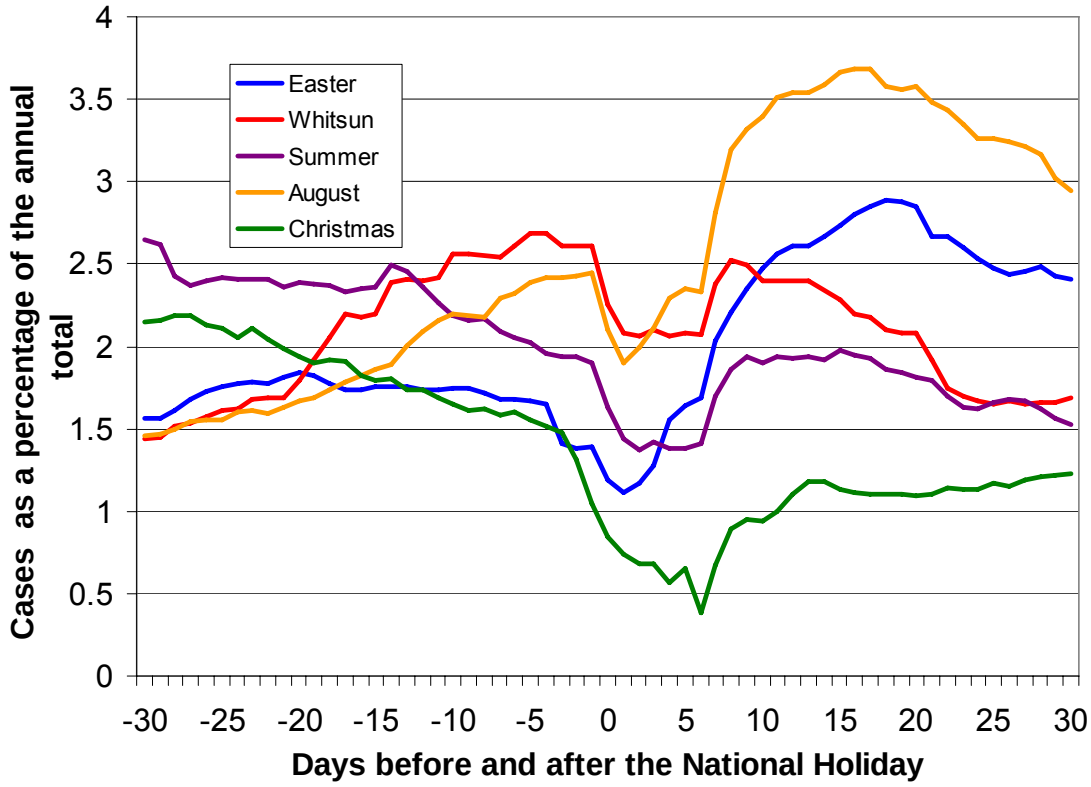
Figure 4. *Cryptosporidium* reporting by day of week 1989-1994



5.7 **Bank Holidays.**

5.8 Surveillance data is influenced by the occurrence of national holidays (Figures 5; Appendix 18 & 19). Analysis of the national data shows a cumulative reduction in cases associated with these holidays. In addition there is an increase in cases following the Easter and August Bank Holidays and a decrease following thelate spring holiday and the Christmas break. It is presumed that the reduction in reporting at these times reflects reduced access to medical services, although it may also be linked to children being off from school. A similar reduction has also been observed for Campylobacter.

Figure 5. The occurrence of *Cryptosporidium* reports before and after National Holidays 1989-2002



5.9 Diagnostic laboratory testing policy.

5.10 Not all hospital laboratories test for *Cryptosporidium* and even those that do carry out such tests will not necessarily do so for all samples. The testing policy is usually generated by the laboratory Microbiologist/Director, although the policy for private laboratories both in England and Wales and abroad is often influenced more by the requesting physician (the client). The HPA has a Standard Operating Procedure for testing *Cryptosporidium* and recommends testing all faecal samples.

http://www.hpa-standardmethods.org.uk/documents/bsop/pdf/bsop31.pdf

5.11 The UK *Cryptosporidium* Reference Unit regularly audits laboratories for their screening and reporting policies and practices A. A survey conducted in North West England and Wales (49 laboratories, 48 responded) in 2000 found that 81% of laboratories screened all samples for *Cryptosporidium* while 19% screened a sub-set of all samples received (Chalmers et al., 2002c). In 2002 the survey was extended to cover the whole of England and Wales (217 laboratories, 187 responded) and it was found that a lower number (53%) screened all samples and figures ranged from 87% for Wales to 25% for London (Table 1). The apparent change was due to the extension of the survey to areas less well covered in terms of full screening than those included in the first survey (Table 4).

Table 1. Details of the percentage of laboratories testing all faecal samples for *Cryptosporidium* in a 2002 survey conducted in England and Wales

Region	Test all specimens for *Cryptosporidium*	Apply selection criteria
Eastern	7/13 (54%)	6/13 (46%)
East Midlands	6/10 (60%)	4/10 (40%)
London	7/28 (25%)	21/28 (75%)
Northern and Yorkshire	13/30 (43%)	17/30 (57%)
North West	20/29 (69%)	9/29 (31%)
South East	13/27(48%)	14/27(52%)
South West	10/17 (59%)	7/17 (41%)
West Midlands	11/18 (61%)	7/18 (39%)
Wales	13/15 (87%)	2/15 (13%)

5.12 In 2002, patient age was the second most frequently applied selection criterion, after immunocompromised patients, included in the testing policy of a laboratory (57 labs). Age criteria were applied by 46 labs, and ranged from patients under 2 to those under 60 years. Nine laboratories did not screen children under 1 year of age. Other selection criteria included: stool consistency (46 labs); duration of diarrhoea (12 labs); history of farm visits (33 labs); foreign travel history (35 labs). When foreign travel was used, this was most often defined as any foreign travel outside the UK (16 labs), sometimes it was not defined (14 labs), and other definitions included outside north Europe (1 lab), outside Western Europe (1 lab) or a specific named location (2 labs). Other selection criteria included 'on clinician's request',10 labs stated that they excluded hospital in-patients, but policy varied regarding duration of stay and onset of symptoms in relation to screening for *Cryptosporidium*.

5.13 The most recent audit is taking place at the present time and covers the whole of the UK (248 laboratories). Questionnaires were distributed in April 2006.

5.14 Sensitivity of laboratory diagnosis.

5.15 The methods used in primary testing laboratories are predominantly based on the staining and microscopic examination of faecal specimens. Auramine phenol stained slides examined by fluorescence microscopy (Nichols and Thom, 1984) is the most common procedure (about 2/3rds laboratories), while modified Ziehl-Neelsen stained slides (Henriksen and Pohlenz, 1981) examined by light microscopy is used in about 1/3rd of laboratories. A recent development since the 2002 survey has been the use off immunologically-based test kits for the detection of *Cryptosporidium* antigens in faecal samples.

5.16 The comparative sensitivity of laboratory methods for detecting the presence of *Cryptosporidium* in faecal samples has been studied recently using archived faeces from a study of Infectious Intestinal Diseases undertaken ten years ago. When samples from both cases and controls were re-tested using molecular

methods the number of *Cryptosporidium* positive samples increased from 27 to 60 (Table 2). This is consistent with information from human volunteer experiments conducted in Texas (Okhuysen et al., 1998). These results imply that laboratories using microscopy (the most common method) are failing to detect about half of all *Cryptosporidium* cases alone.

Table 2. Results of the detection of *Giardia* and *Cryptosporidium* by conventional microscopy and PCR techniques (n= number of samples tested) from IID samples (projects B14004/5 funded by the Food Standards Agency)

			Number of samples in which the following agent was detected							
			Giardia				*Cryptosporidium*			
		Specimens n =	Micro-scopy	Microscopy +PCR	(%)	p value, methods	Micro-scopy	Microscopy +PCR	(%)	p value, methods
All	cases	2422	24	45	(2)	0.01	27	60	(2)	<0.01
	controls	2205	11	32	(1)	<0.01	2	12	(0.5)	<0.01

5.17 **Completeness of demographic reporting details.**

5.18 A positive finding of *Cryptosporidium* is reported by the laboratory to the patient's physician, the local Consultant in Communicable Disease Control, the local Environmental Health Department and nationally to HPA surveillance. The reporting of *Cryptosporidium* cases should carry with it anonymised details of the patient, including age, sex, postcode, etc. The completeness of the additional information has changed for the better over time as electronic reporting sytems have been introduced (Figure 6-8) but in some cases it has deteriorated. Where such information is not supplied in a timely manner the ability of national surveillance to detect and respond to outbreaks is reduced.

Figure 6. Changes in *Cryptosporidium* reporting delay 2001-2005

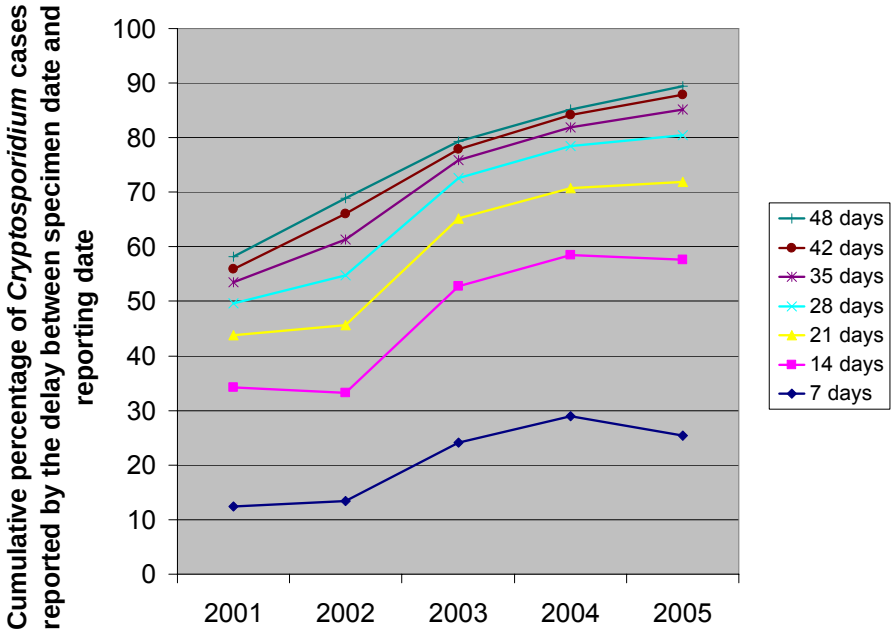


Figure 7. Percentage of *Cryptosporidium* cases without a reported age

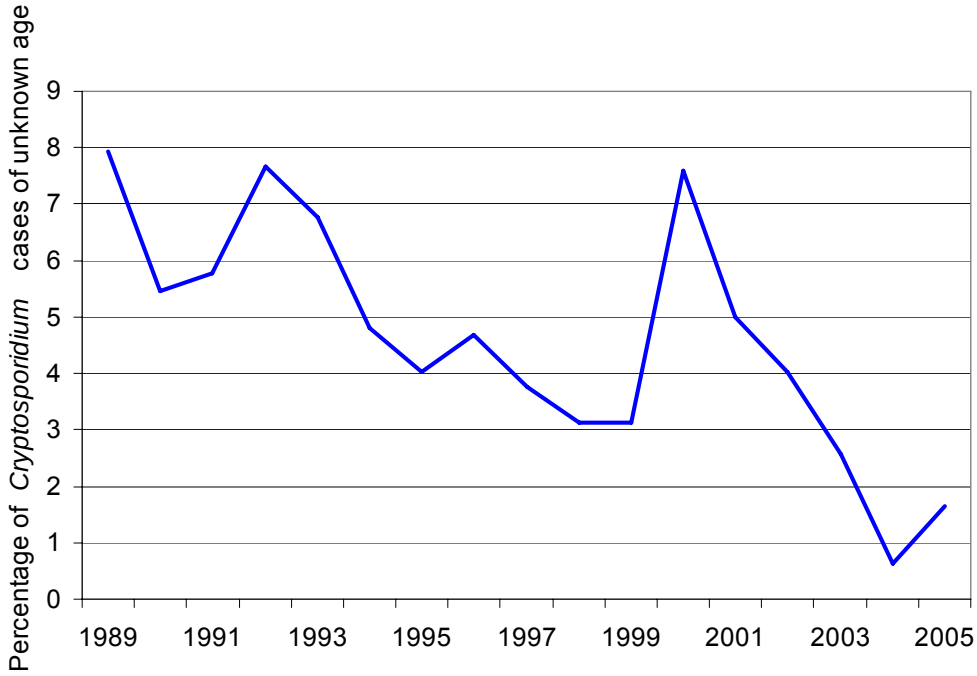
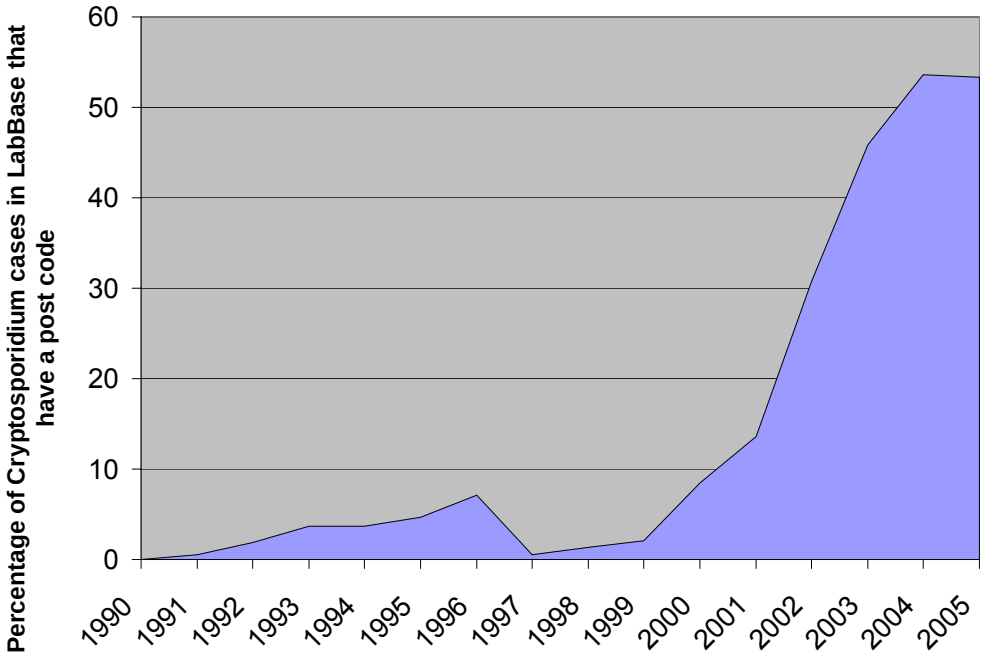


Figure 8. Percentage of *Cryptosporidium* reports with a post code



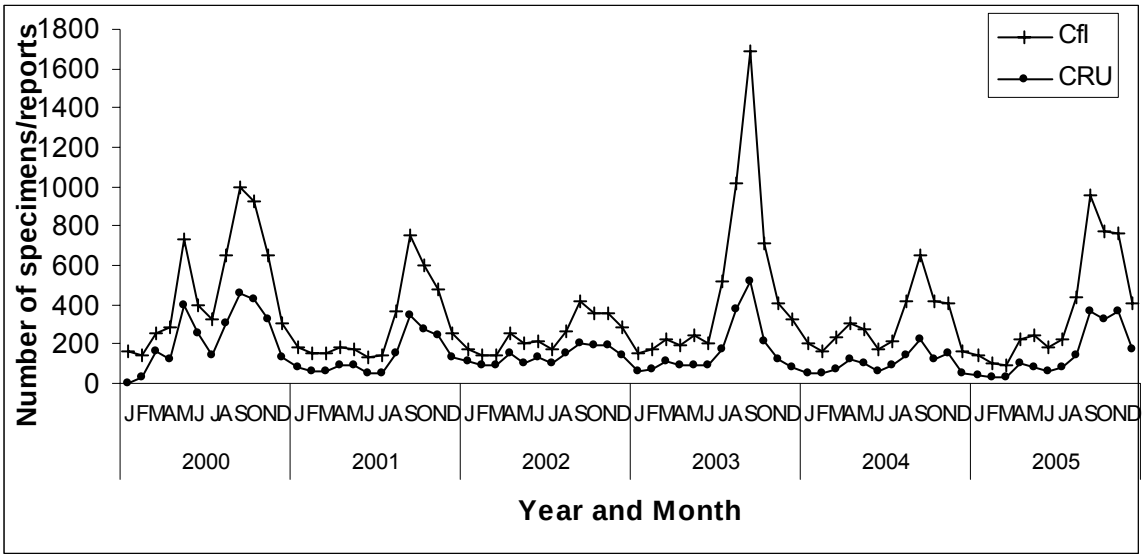
5.19 **Genotyping of positive specimens.**

5.20 Laboratories submit faecal samples to the UK *Cryptosporidium* Reference Unit for confirmation of the finding and for differential diagnosis of *Cryptosporidium*.

5.21 *Cryptosporidium* typing was first conducted on a population basis through a collaboration between the Food Safety Microbiology Laboratory in Colindale and the *Cryptosporidium* Reference Unit (CRU) which was then located in Rhyl (Patel et al., 1998; McLauchlin et al., 1998) following developmental work in the late 1980s and early 1990s (Nichols et al., 1991; Nichols, 1992). This work lead to population based typing studies and examination of outbreaks of *Cryptosporidium* in the late 1990s (McLauchlin et al., 2000; McLauchlin et al., 1999). In 2000 the CRU relocated to Swansea.

5.22 Between January 2000 and December 2003, all laboratories in England and Wales were asked to send all *Cryptosporidium*-positive faecal samples to the CRU for genotyping to species level as part of a project co funded by DWI and Scottish Executive to create a national collection of *Cryptosporidium* oocysts (Scottish isolates were sent to the Scottish Parasite Diagnostic Laboratory). This generated 8075 specimens from England and Wales, representing 44% of the number reported to national surveillance over the same time period. However, since specimens in the latter study were anonymised it is impossible to establish if the UK CRU collection of specimens is reflective of the whole population. Nonetheless, the collection has been shown to be representative of national surveillance in terms of age, sex and monthly distribution (Figure 9) (Chalmers et al., in preparation).

Figure 9. Number of cases reported to national surveillance (CfI) and number of isolates sent to CRU for typing, 2000 to 2005



5.23 The PHLS carried out a case control study of sporadic cases of cryptosporidiosis occurring in 2001 and 2002 funded by DWI (Hunter et al., 2004) as well as further investigation of subtyping methods (Chalmers et al., 2005).

5.24 Since January 2004 routine genotyping to the species level has been conducted on a sentinel basis of 43 specifically identified laboratories throughout England and Wales, to support projects (Investigation of *Cryptosporidium* clinical isolates and analysis with epidemiological data funded by DWI and Evaluation and risk assessment of zoonotic transmission of *Cryptosporidium* funded by DWI and Defra).

5.25 In addition to the sentinel scheme, any laboratory can send isolates for genotyping to the species level to support outbreak/cluster investigations.

5.26 Sub-typing has been funded by DWI to investigate trends and risk factors with *C. parvum* and *C. hominis* in cases from the case control study, and to further investigate the role and value of *C. hominis* typing for epidemiological purposes.

5.27 Improved methods for the analysis of environmental samples and recovery of DNA were developed at the UK CRU as part of a PhD studentship (Guy Robinson 2005).

5.28 Typing of environmental samples has also been undertaken as part of research (Establishing the relationship between farm re-stocking and cryptosporididia: the Caldew catchment study Parts 1 and 2, funded by UKWIR and Defra) and for public health purposes, largely to inform outbreak control teams.

5.29 National typing.

5.30 Molecular microbiology has provided a valuable tool to enable trends to be observed for infections caused by *C. parvum* and *C. hominis* Appendix 8. Analytical epidemiology has shown statistically significant differences between these two species and significant species-specific risk factors (Hunter et al., 2004).

5.31 A decision on whether to type all *Cryptosporidium* positive samples in future needs to be made on the basis of how it might inform risk based decision making by those responsible for public health and water supply management. Similarly, a decision on the extent to which sub-typing is required however first the sub-typing methods need formal evaluation. Funding for species-level typing has been made available for laboratories in Scotland as part of the reference laboratory service provided by the UK *Cryptosporidium* Reference Unit. The cost benefit of extending this approach in England and Wales will be informed by the work in Scotland.

6 Epidemiology of cryptosporidiosis

6.1 History

6.2 Symptomatic cryptosporidiosis was first noted in turkeys in 1955. During the 1970s *Cryptosporidium* infections were reported to cause neonatal diarrhoea in calves. The first human cases of cryptosporidiosis in humans were recorded in the 1970s; one in a young girl with enterocolitis (Nime et al., 1976) and the other in an AIDS patient (Meisel et al., 1976). With the spread of AIDS in the 1980s and 1990s more and more cases of cryptosporidiosis were diagnosed (Dillingham et al., 2002). Cryptosporidiosis occurs worldwide. Sero-epidemiological studies have indicated that 20% of Americans have experienced infections, while in Chinese children the number who appear to have been exposed reaches 65% (Dillingham et al., 2002).

6.3 In immunocompetent patients, the symptoms of cryptosporidiosis can include; watery diarrhoea, abdominal cramping, mild fever, nausea and vomiting, headaches, fatigue, anorexia, and respiratory problems. Diarrhoea is the most common symptom, and results in the shedding of large numbers of infectious oocysts. The incubation period is around seven days and symptoms typically last for one or two weeks with oocysts being present in faeces for around 18 days after the onset of symptoms (occasionally for as long as 50 days). Asymptomatic infections are also recorded with the faecal shedding of infectious oocysts while feeling well. (Arrowood 1997)

6.4 As well as the acute symptoms there are potential long-term consequences of *Cryptosporidium* infections. Infection at a young age can lead to impaired development and growth, and possible long-term cognitive deficits, especially among children in the developing world. (Dillingham et al. 2002)

6.5 In patients with a suppressed immune system (resulting from AIDS or therapy following transplant surgery) the consequences of infection with *Cryptosporidium* are severe and sometimes fatal. The symptoms of cryptosporidiosis occur for prolonged periods of time, and infection can spread beyond the intestinal tract to sites such as the gall bladder or bile duct. Patients can make a full recovery, especially if immunity is restored (e.g. by cessation of immunosuppressive therapy, or treatment for malnutrition), but some do not.

6.6 Cryptosporidiosis was first recorded in England and Wales in the 1970's and screening of faecal samples began in 1983 (Figure 10). The years between1983 to 1988 were characterised by an increase in cases as a result of greater laboratory detection, screening and reporting. The peak in cryptosporidiosis in 1989 was due mostly to the large waterborne outbreak in Oxford/Swindon. Since then the *Cryptosporidium* cases have remained between 3000 and 6000 per year (mean 4559). The pattern of distribution is characterised by many short spikes which differs from the pattern for other enteric pathogens, emphasising that the distribution of *Cryptosporidium* cases is strongly influenced by both detected and undetected outbreaks (Figure 12).

Figure 10. *Cryptosporidium* cases per year 1983-2005

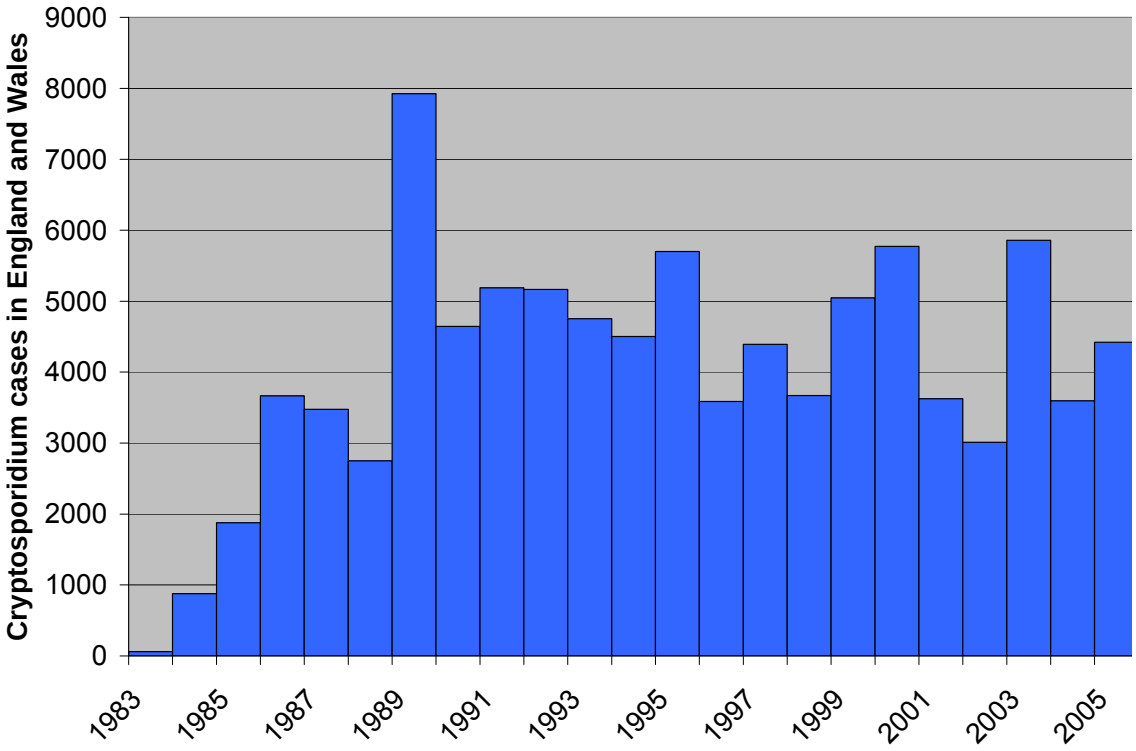
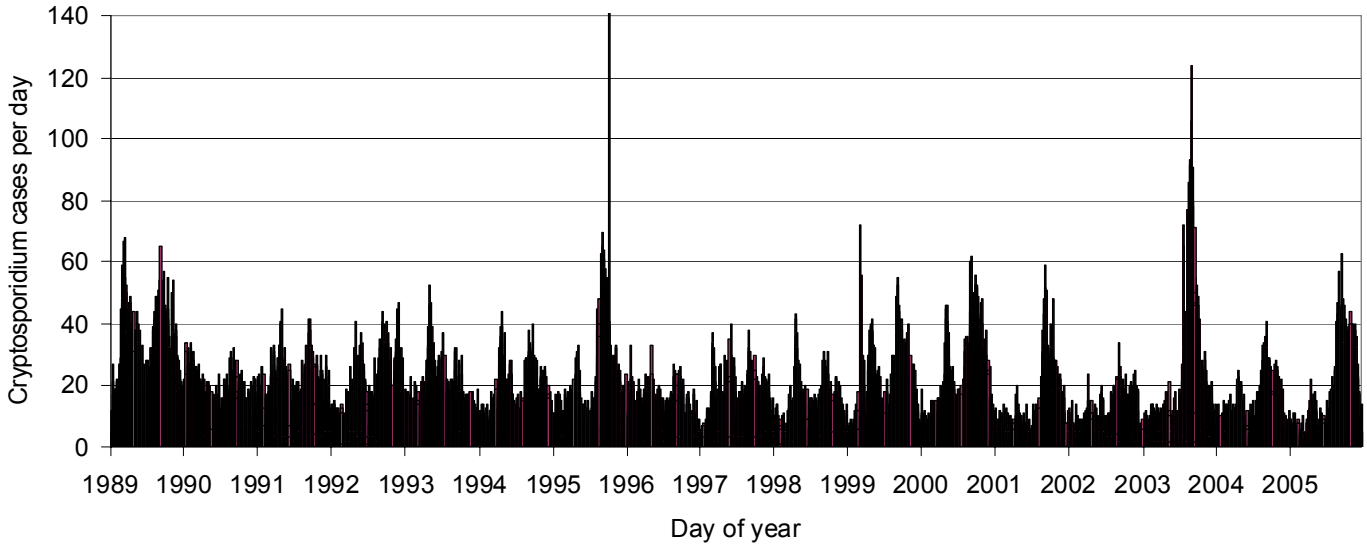


Figure 11. *Cryptosporidium* cases by day 1989-2005



6.7 The data shows a seasonal distribution with more cases in the spring and autumn than in summer and winter between 1991 and 2000. It is likely that this seasonality pattern holds true both for the occurrence of outbreaks and sporadic

disease. The spring increase in cases has declined substantially since 2001 with the reduction being most striking in the North West Region.

6.8 The prevalence of cryptosporidiosis can be estimated by measuring antibody levels in people within the population and these are called seroprevalence studies. Seroprevalence studies can be conducted using a variety of techniques for antibody detection. These studies are hampered by the current lack of consensus of cut-off values for positivity, particularly in Western blot tests, where intensity of antibody response gives a reliable outcome for seroepidemiology. Three epidemiological studies are underway at the *Cryptosporidium* Reference Unit, where a min-blot system has been validated with sera from diagnosed cases of cryptosporidiosis in a time series study:

1. paired serum study (comparing the seroprevalence and incidence in a town with a high reported incidence of cryptosporidiosis with a town with a low incidence of cryptosporidiosis
2. intervention study, comparing pre-and post-water treatment intervention with a comparison area
3. A study of a cohort of farmworkers that is examining exposure to a range of zoonotic pathogens including *Cryptosporidium*

The laboratory analyses have been completed at the CRU and the statistical analysis is due for completion later this year.

6.9 Incidence and prevalence

6.10 The prevalence of a disease can be defined in a number of ways but for an infectious disease this is usually expressed as the number of cases per 100,000 population per year. The rate of disease may differ by age and geography and this information may reflect outbreaks, differences in disease ascertainment and other factors. The reliability of population estimates derived from census data, is another influencing factor particularly due to under representation of immigrant and transient populations. Incidence can be used to express both a percentage of the population affected or a percentage of faecal samples tested that were positive and prevalence is generally preferred over incidence when talking about populations.

6.11 Geographic differences in prevalence

6.12 The prevalence of cryptosporidiosis increased between 1983 and 1989 as a result of improved laboratory detection. From 1989 onwards the geographic differences have been strongly influenced by outbreaks

6.13 The prevalence of cryptosporidiosis differs by Region with rates varying by region for the years 1989-2004 from 1.9 to 23.9 with a mean of 8.9 / 100,000 / yr (Table 3). There have been notable reductions in the North West from 14.6 / 100,000 / yr between 1990 and 2000 to 8.4 / 100,000 / yr for the years 2001 to 2004 (a 42% reduction). There have also been reductions of between 7 and 24% over this time period in seven other regions but an increase in the West Midlands of 5% and in the Eastern region of 25%. The prevalence was lower in 2001 and 2002 than in

2000 or from 2003 to the current time, suggesting a specific cause of the reduction in these two years. The most probable explanation was the Foot and Mouth Disease outbreak which significantly reduced livestock grazing (Hunter et al., 2003; Smerdon et al., 2003). Some of the effect is also likely to have been as a result of the bringing on line filtration of water supplies in the North West that had not previously been filtered (Sopwith et al., 2005). Throughout these data ascertainment may contribute to overall regional differences.

Table 3. Prevalence of cryptosporidiosis per 100,000 population per year by region (all ages)

Laboratory Region	1990	1991	1992	1993	1994	1995	1996	1997	1998	1999	2000	2001	2002	2003	2004
East Midlands	6.3	8.7	8.3	7.7	9.7	9.3	6.0	8.6	7.3	8.4	11.7	6.4	6.3	11.8	6.5
Eastern	3.8	7.9	9.4	9.4	6.7	12.4	5.1	11.4	5.1	7.6	9.3	8.5	6.6	12.3	12.7
London	2.3	7.1	3.4	2.3	2.3	3.4	2.3	2.3	1.9	3.0	3.9	2.6	2.0	3.6	2.8
North East	10.1	5.8	5.0	5.9	7.0	4.7	13.4	5.8	4.1	7.3	8.8	5.6	5.0	11.0	4.8
North West	9.2	12.3	18.9	13.9	11.5	12.5	10.8	16.2	12.7	21.1	21.4	7.8	8.2	11.0	6.7
South East	9.6	12.6	7.2	8.6	9.0	10.7	5.9	6.8	6.1	7.1	8.8	6.9	5.5	10.1	5.0
South West	14.5	15.4	14.3	12.2	12.8	22.2	9.0	10.3	10.3	12.9	14.9	8.8	7.5	16.8	10.0
West Midlands	7.3	6.5	7.7	8.7	4.9	7.7	6.4	5.7	5.6	8.7	10.4	7.4	4.8	12.0	6.3
Wales	6.8	12.1	11.4	12.1	11.6	12.6	8.0	7.3	9.4	11.7	12.5	8.5	6.1	11.0	6.2
Yorkshire & Humberside	23.9	12.0	14.9	13.1	14.9	16.2	7.6	9.8	9.0	9.8	11.0	8.8	6.5	16.8	8.3

6.14 When the prevalence is examined using a two-sample t test with unequal variances the reduction in cryptosporidiosis between the periods 1990-2000 and 2000-2004 was statistically significant for the North West region (p=0.002) but not for each of the other regions.

6.15 Cryptosporidiosis prevalence has ranged from 0.1 / 100,000 / yr to 47 / 100,000 / yr, with an average of 8.7 / 100,000 / yr when examined by Strategic Health Authority (Appendix 9). The prevalence in children is likely to give the best estimate of exposure to infection because ascertainment is better in this group and prior infection is less likely (Appendix 10, Figures 12 & 13)).

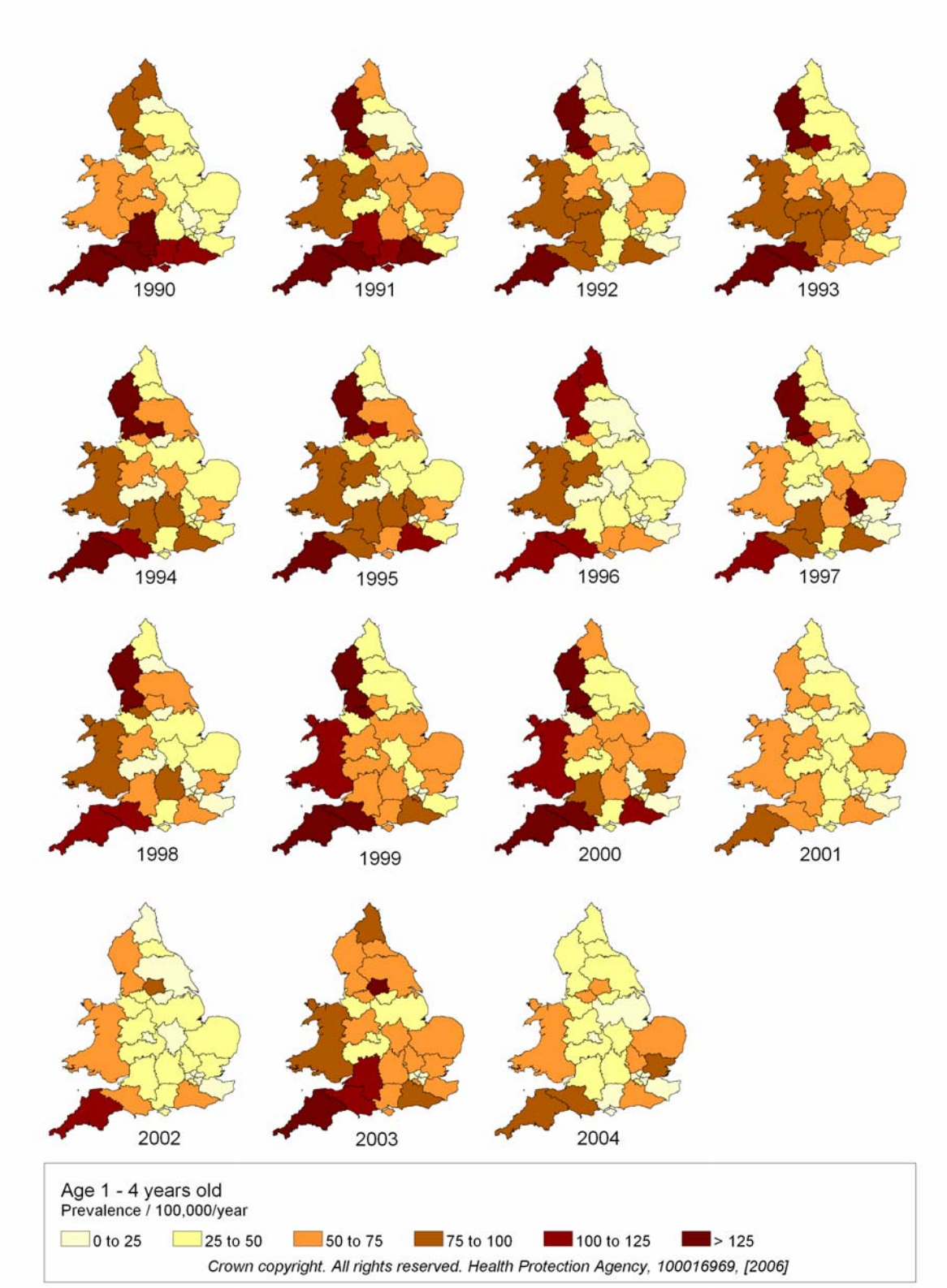
6.16 Geographic differences in prevalence in children

6.17 Cryptosporidiosis rates may differ between populations on the basis of their long-term exposure to oocysts and subsequent full or partial immunity. It is therefore possible to look at the underlying exposure in more detail by examining the prevalence in the 1-4 year age group who are likely to have had less prior exposure to infection than adults. This population is also likely to have better than average ascertainment because the laboratory screening criteria discussed in 5.5 are likely to include this age group for most laboratories. (Figure 12). Examination of data from Wales and the 28 Strategic Health Authorities in England has identified substantial regional differences in prevalence in this age group (Appendix 10). For this group the highest prevalence for the period 1990 to 2000 is in Combria & Lancashire, Greater Manchester, the South West peninsula, North And East Yorkshire and Somerset and Dorset.

6.18 There have been significant reductions in prevalence in 1-4 year olds in the Greater Manchester and Cumbria and Lancashire Strategic Health Authority areas in the period since the start of 2001 which is thought to reflect the introduction of water filtration and other measures designed to reduce source water contamination. The low prevalence in London probably reflects historically poor rates of reporting that have yet to be properly addressed. There has been a reduction in prevalence in Bedfordshire and Hertfordshire following the outbreak in 1997. Other than these observations, the prevalence in the 1-4 year age group seems not to have substantially changed in recent years.

6.19 Two-sample t test with unequal variances was performed on years 1990 to 2000 and 2001 to 2004 to test for significant differences between these year periods for Strategic Health Authority (Appendix 10). There were significantly lower prevalences in the period 2001-2004 compared with the period 1990-2000 for Cumbria & Lancashire ($p=0.00001$), Greater Manchester ($p=0.0007$), Thames Valley ($p=0.0008$), South West Peninsula ($p=0.03$), Dorset & Somerset ($p=0.01$), and Wales (0.02). For other Strategic Health Authorities the differences were not significant.

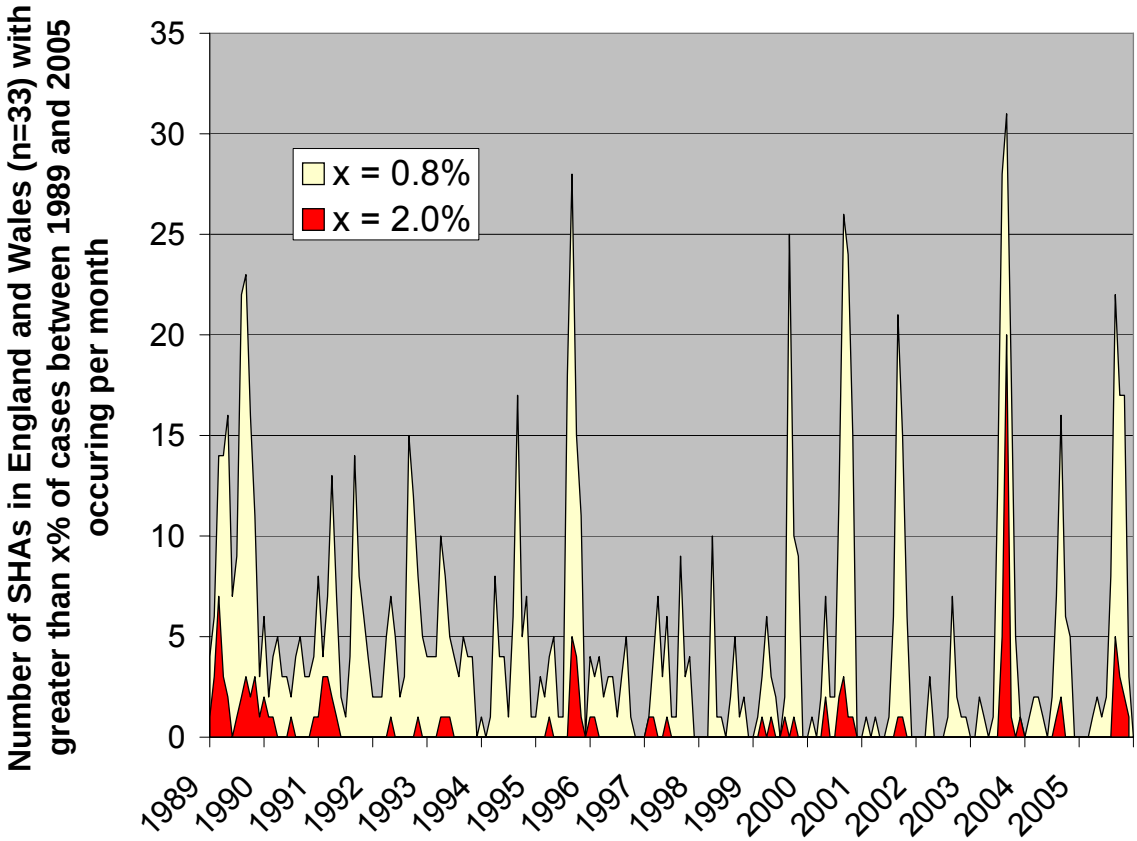
Figure 12. Prevalence of cryptosporidiosis by Strategic Health Authorities in Children aged 1-4 years old (cases per 100,000 population per year)



6.20 **Geographic differences over time**

6.21 Examination of the data by Strategic Health Authority area (SHAs) in England and Wales by month shows several occasions where there has been an increase in cases across most SHAs (Figure 13), that could represent evidence of the impact of a common source of infection. Some of these increases are co-incident with a swimming pool outbreak in hotel in Majorca in 2003, and the outbreak in Torbay in 1995. While this change across the country could be seasonal and influenced by behaviour (such as altered swimming pool use), it could represent a common source affecting people across the country (such as travellers having visited a resort where an outbreak is occurring). However there is also an underlying national trend for an increase in prevalence in the late summer / autumn in years 1999, 2000, 2001, 2003 and 2005 that is in need of investigation.

Figure 13. The number of SHAs with greater than x% of all cases within the period 1989-2005 occurring per month (average cases per month 0.49%)



6.22 **Disease burden**

6.23 There is considerable uncertainty about the percentage of the total number of cases of cryptosporidiosis in a community that are reported to national surveillance. Data from a study of infectious intestinal diseases (IID) estimated that only 1 in every 7.4 cases of cryptosporidiosis in 1995 appeared in national surveillance (Adak et al., 2002). However, it must be borne in mind that such multipliers are very crude and not stable over time. Additionally this approach does not take account of geographically defined outbreaks.

6.24 Recent re-analysis of the samples used in the IID study using molecular methods indicates that using conventional microscopy alone laboratories are missing about the same number of cases as they are detecting (unpublished data from a Food Standards Agency project). However, even this figure may be a substantial underestimate as not all cases of cryptosporidosis are stool positive especially in people with partial immunity (Chappell et al., 1999) and a recent paper has suggested that a substantial proportion of culture negative diarrhoea may be due to cryptosporidioisis (Frost et al., 2005). It is therefore likely that the numbers of cases reported to national surveillance are an order of magnitude smaller than the actual number in the community.

6.25 The best estimate on available evidence is that there is likely to be around 15 cases within the community for every case diagnosed which translates to an average of around 64,000 *Cryptosporidium* cases per year (range 45,000-88,000) over the last ten years, compared to the average of cases detected of 4322 (range 3037-5923).

6.26 **Medical Treatment**

6.27 In people with an intact immune system, cryptosporidiosis is an unpleasant but self-limiting gastroenteritis, although symptoms may be prolonged and can persist for two weeks or sometimes longer. Diarrhoea may be accompanied by vomiting, abdominal cramps and loss of appetite. In the immunosuppressed, especially those with low numbers of T-cells (such as is seen with HIV) or defects in T-cell operation (as in some congenital immunodeficiencies), symptoms are more severe and the whole gastrointestinal tract including the gall bladder, pancreatic duct and even the bronchial tree can be affected. These patients frequently experience chronic or intractable disease.

6.28 Treatment options for *Cryptosporidium* are limited, and this is an important reason why prevention and risk reduction are the most important public health interventions for this particular disease. Treatment, if given, may consist of antiparasitic drug therapy, or drugs that repair or stimulate the immune system.

6.29 **Immune reconstitution**

6.30 For those with an HIV infection, the modern anti-retroviral treatment regimens (HAART) are the treatment of choice. As well as restoring a degree of immune response, some of these agents have a direct anti-parasitic effect . In other types of immunosuppression such as bone marrow transplantation, improving immunity can also lead to improvement of cryptosporidiosis.

6.31 Drug treatment

6.32 No drug treatments are licensed in the UK for treating patients who have a *Cryptosporidium* infection. Those drugs which have been used in the UK include paromomycin, spiramycin, azithromycin and clarithromycin, which all have anti-parasitic activity. There are anecdotal reports of success particularly with paromomycin alone or in combination with azithromycin, but these are not backed up by convincing clinical trial data. Paromomycin may also enhance the benefit of HAART in HIV positive patients.

6.33 In the US, nitazoxanide was licensed by the Food and Drug Administration in 2002 for use in immunocompetent children between the ages of 1-11 years. It is marketed as Alinia by Romark Laboratories. The drug is also in widespread use in Latin America, but the manufacturers have not to date submitted an application for licensing in the UK (Romark pharmaceuticals, personal communication). Data support the efficacy of nitazoxanide in the immunocompetent, whilst in the immunosuppressed there is also some evidence of benefit, although it has not been shown to be effective in the subgroup with the most advanced HIV disease. Nitazoxanide is well-tolerated and has a good safety profile.

6.34 Descriptive epidemiology

6.35 Data included is from England and Wales and is stored in the Oracle database called LabBase2 for the period 1989 to 2005. The 2005 data is subject to the reporting delay outlined in 4.4 and must be regarded as provisional.

6.36 Age distribution

6.37 Cryptosporidiosis is a disease of young animals and also particularly affects children under five (Figure 14;15). *Cryptosporidium* cases in England and Wales are more commonly reported in male children than female ones and this holds for babies from one-week old to 16 year old children (Figure 15). The reason for this difference is unclear. Although boys are more active this is unlikely to explain the increased number of cases in babies of a few weeks old. There could be differences in the way parents treat their male and female children during illness, or there could be physiological differences that result in more severe illness in male children than females. The peak in the male / female ratios in young teenagers suggests more risky behaviour amongst boys may be an important contributory factor. For all older age groups there are more cases in females, with this being most pronounced in women of childbearing age. This may reflect greater exposure of women to infected children or a greater tendency of women to go to the GP if they are sick. The low

male / female ratio in the elderly probably reflects the greater longevity of women compared to men.

Figure 14. *Cryptosporidium* age distribution 2000-2005

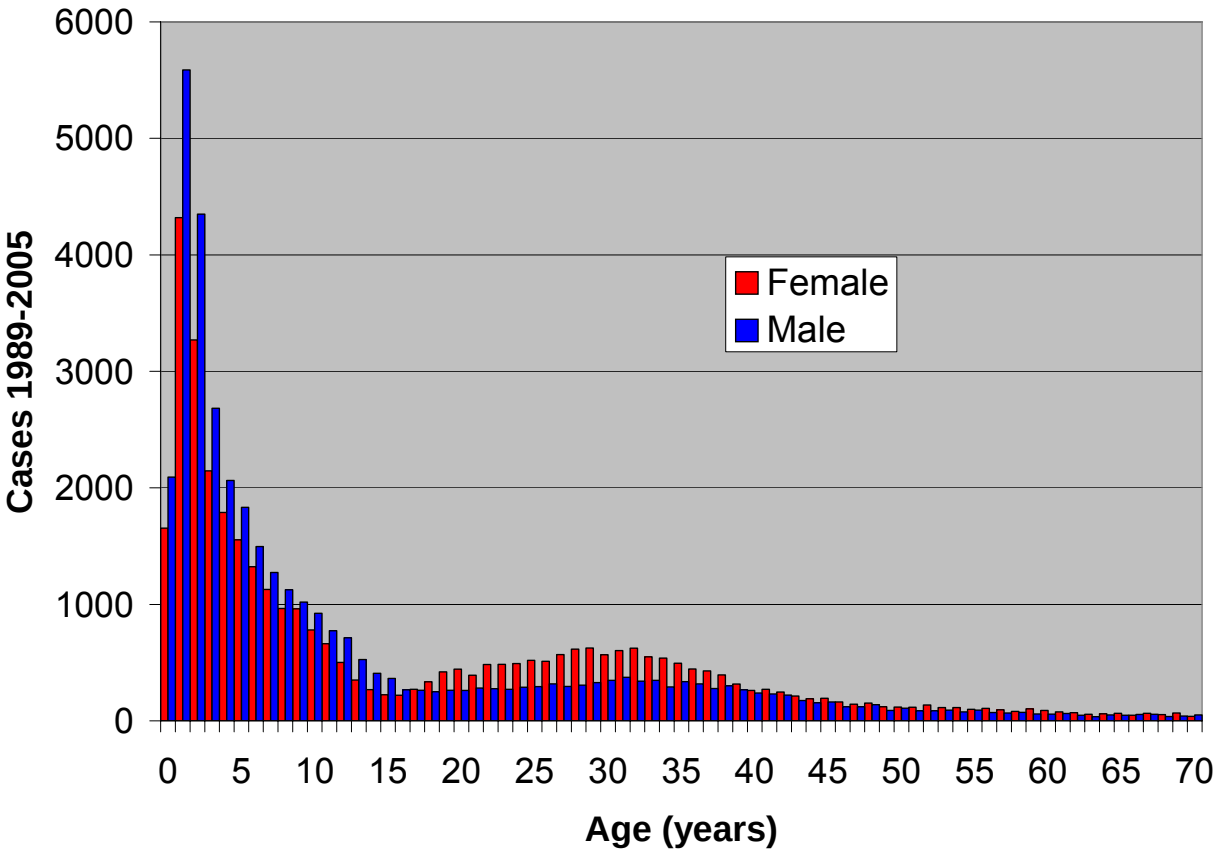
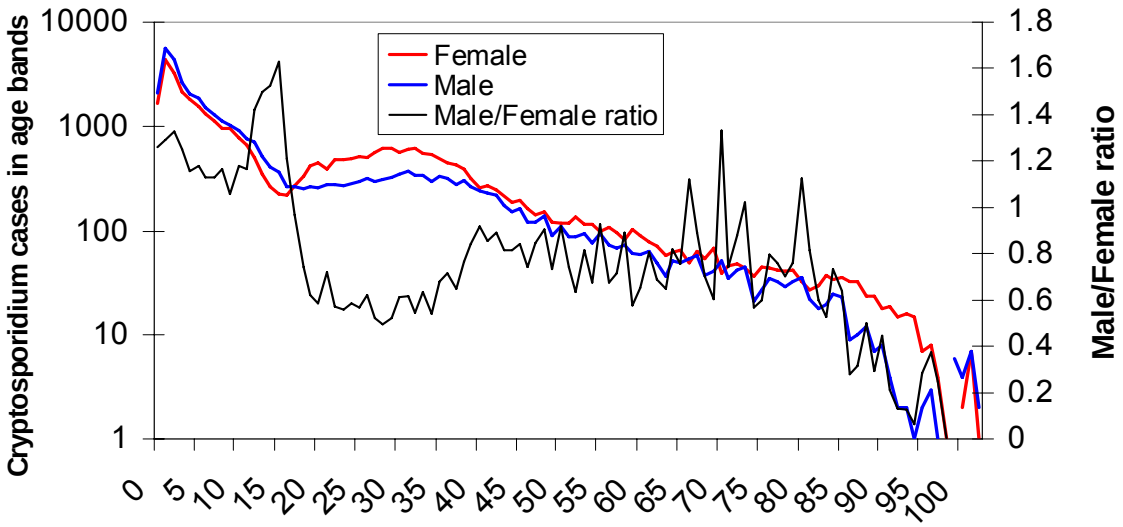


Figure 15. *Cryptosporidium* age and sex distribution 2000-2005



6.38 Changes in age distribution over time

6.39 There have been changes in the age distribution over time, with a long-term decline in the percentage of cases under five and an increase in the percentage in older age groups (Figures 16 - 18). This changed trend may reflect an increase in *Cryptosporidium* testing of faecal samples in all age groups by diagnostic laboratories. A survey of laboratory practice in the north west of England and Wales in 2000 found that over 80% of laboratories were testing all faecal samples for *Cryptosporidium* (Chalmers et al., 2002c). However, the trend may also reflect a reduced risk in the under 5 age group due to reduced exposure to one or more sources.

Figure 16. Change in the percentage of *Cryptosporidium* cases in different age groups 1989-2005

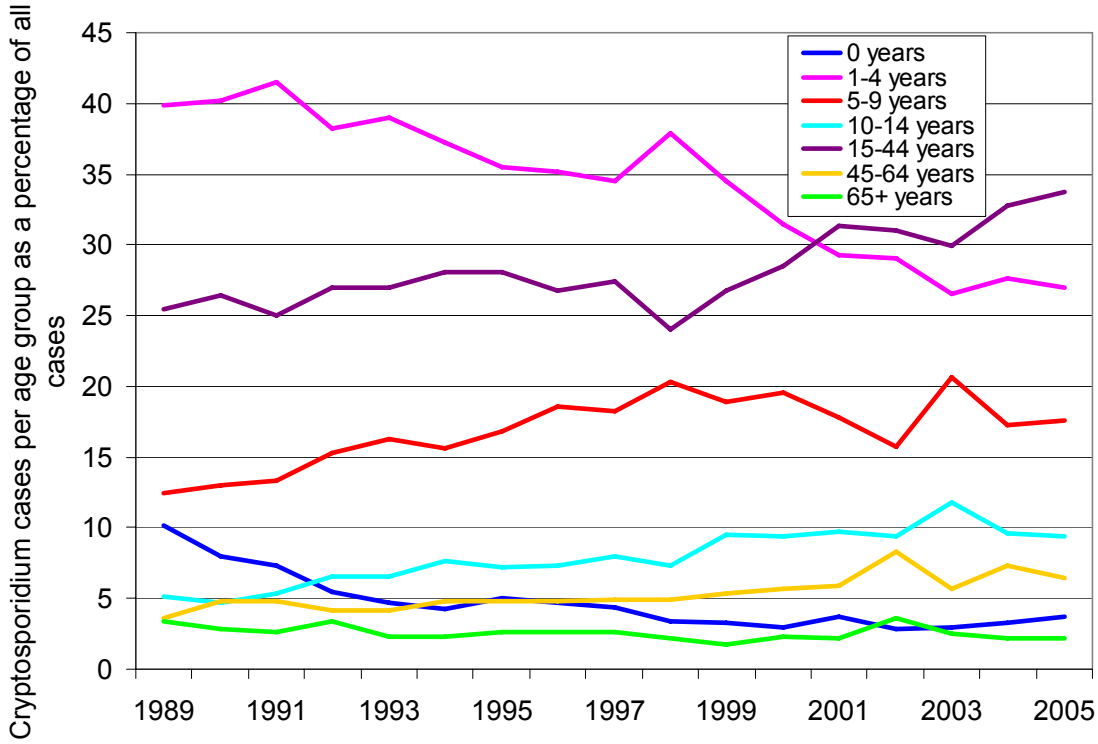
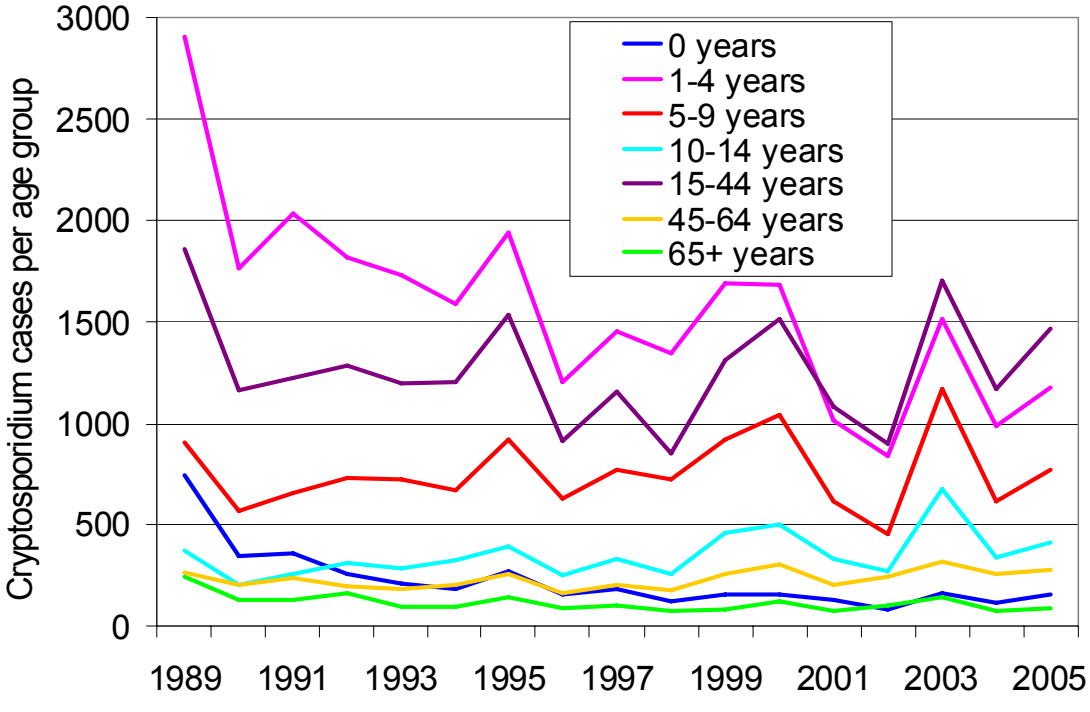
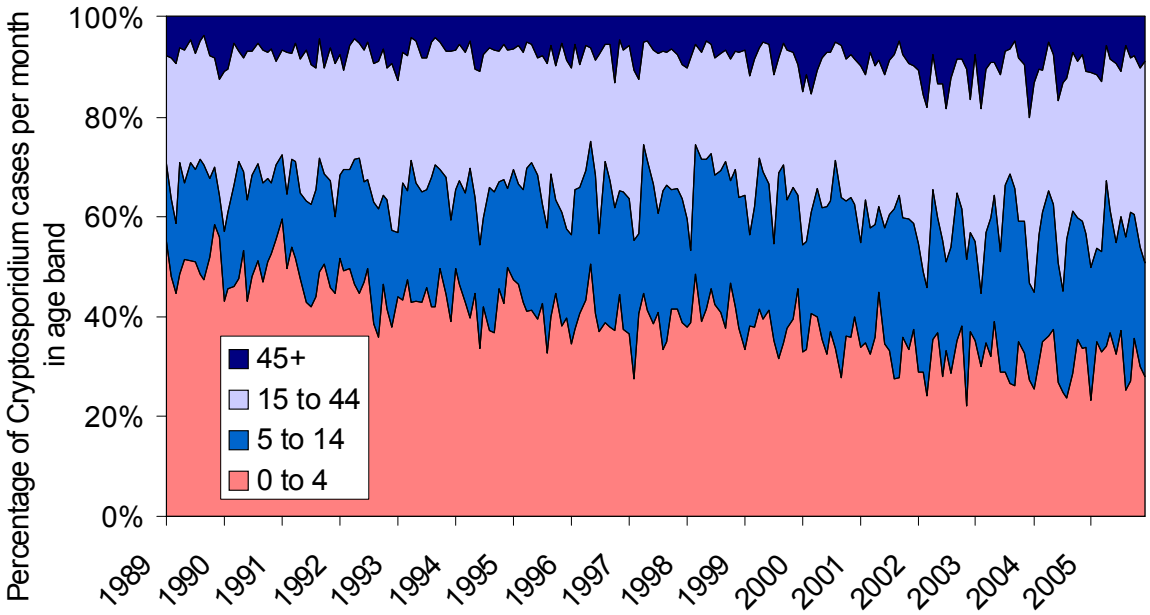


Figure 17. Change in the *Cryptosporidium* cases in different age groups Short term changes in age distribution



6.40 There are changes in the age distribution of cryptosporidiosis over short timescales (Figure 18). These were thought to be due in some way to the impact of outbreaks but there is currently little analytical evidence that such trends can be used for predicting outbreaks.

Figure 18. Change in the *Cryptosporidium* cases in different age groups



6.41 Seasonality of cryptosporidiosis

6.42 Cryptosporidiosis in England and Wales has a strong seasonal pattern, with increased number of cases in spring and autumn (Figure 19). There is an increase in cases between weeks 15 and 21 and another increase beginning around week 31, reaching a maximum at week 36 and subsequently declining through to the end of the year. The data in Figure 16 includes the decreases in cases around Bank Holidays outlined in Figure 5. The seasonality of cryptosporidiosis can be seen to have altered within the period 1989-2005, with large outbreaks in the early part of the year being prominent between 1989 and 1992 in days 1 to 85, but less so in most subsequent years (Figure 20; Table 4). The spring increase tends to occur after Easter through to the summer, but it has declined since 2001, although it has not completely disappeared. The late summer increase usually occurs after the August Bank Holiday and has increased since 2001.

Figure 19. Changes in *Cryptosporidium* cases per day of year using a seven-day rolling mean averaged over four year periods

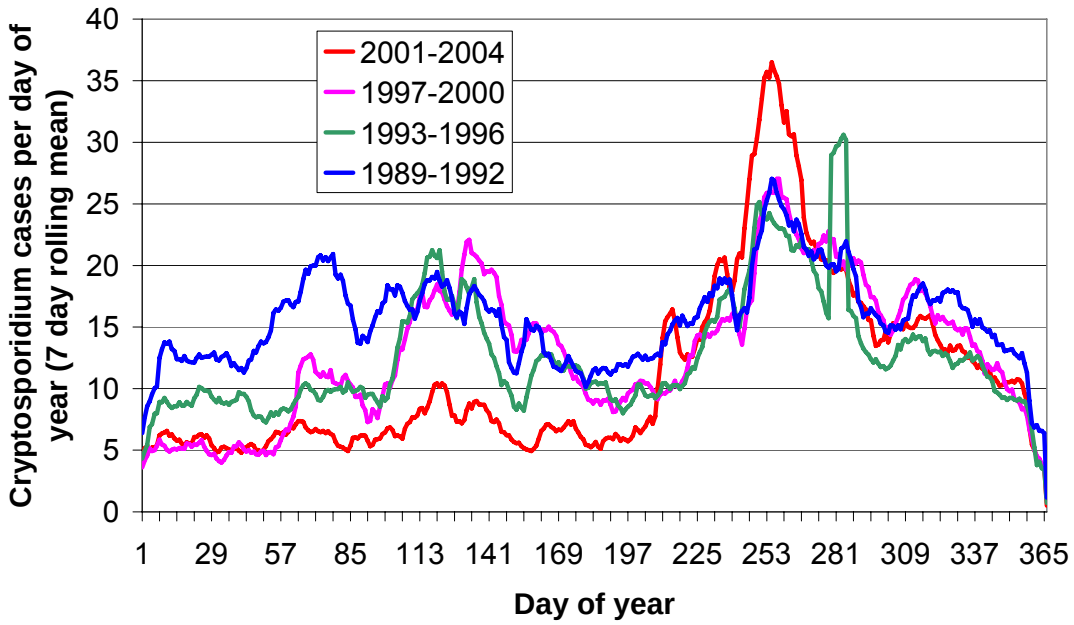


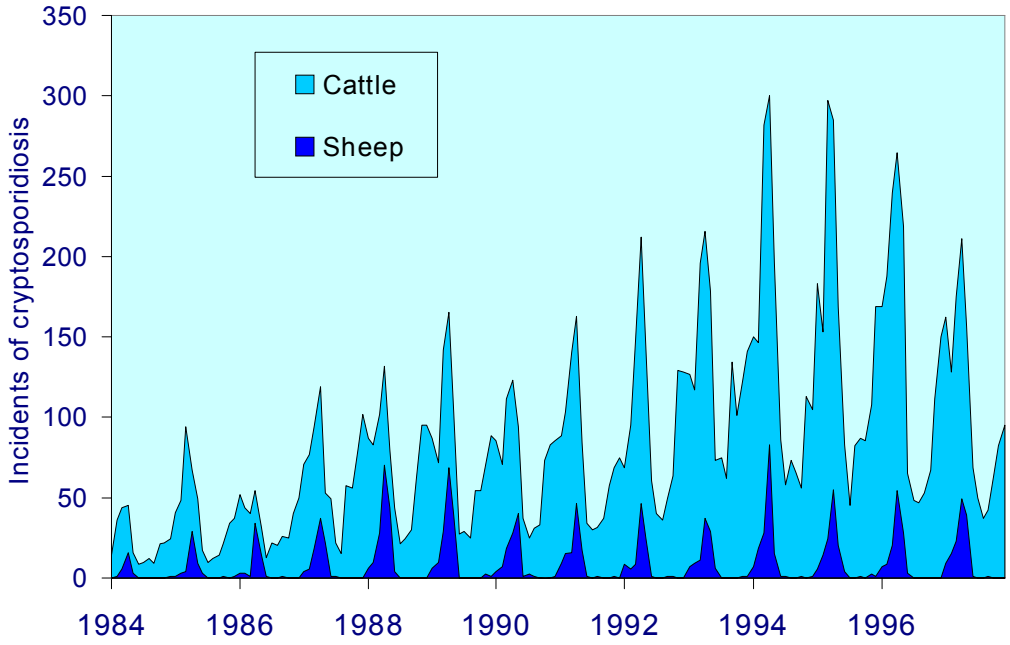
Table 4. *Cryptosporidium* cases by four-year periods

Time period	Jan to June	Jul to Dec	Grand Total
1989-1992	11318	12324	23642
1993-1996	8667	10517	19184
1997-2000	8003	11036	19039
2001-2004	4958	11763	16721
Grand Total	32946	45640	78586

6.43 Seasonal distribution of animal disease

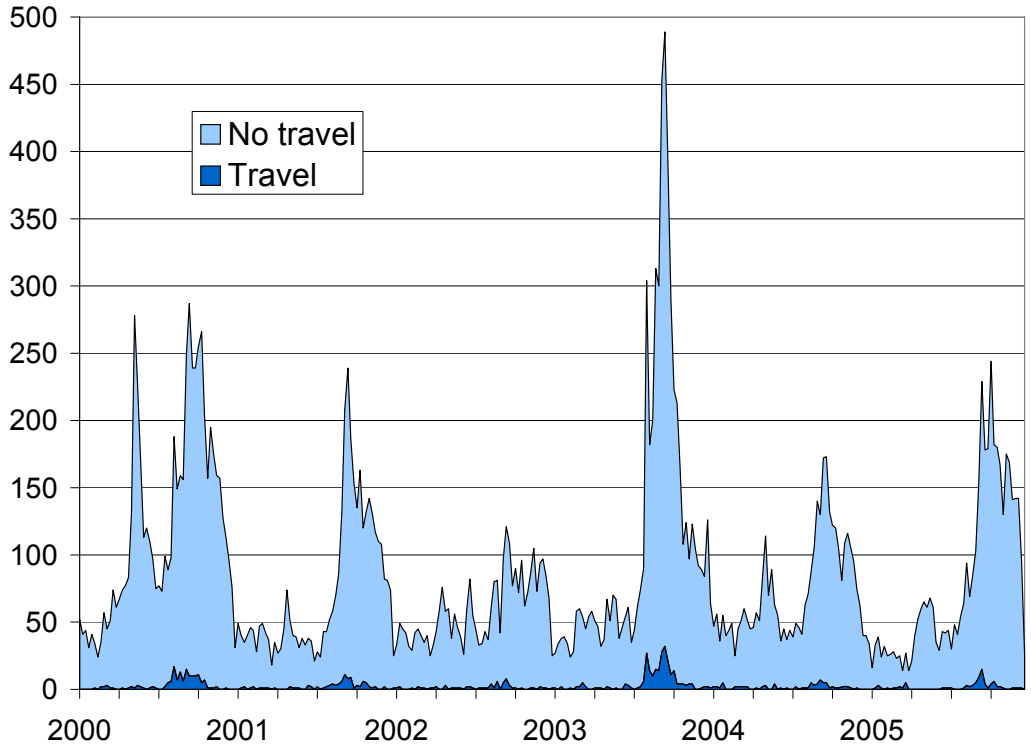
6.44 The regular occurrence of cryptosporidiosis incidents in cattle and sheep farms is linked to disease in lambs and calves. Newborn animals are more susceptible to infection with cryptosporidiosis and scours (diarrhoea) is common in these animals. Accordingly cryptosporidiosis in cattle and sheep occurs predominantly around the periods of lambing and calving, with a strong spring peak in sheep and cases in cattle in both spring and autumn (Figure 20).

Figure 20. Incidents of cryptosporidiosis in cattle and sheep in the UK
6.45



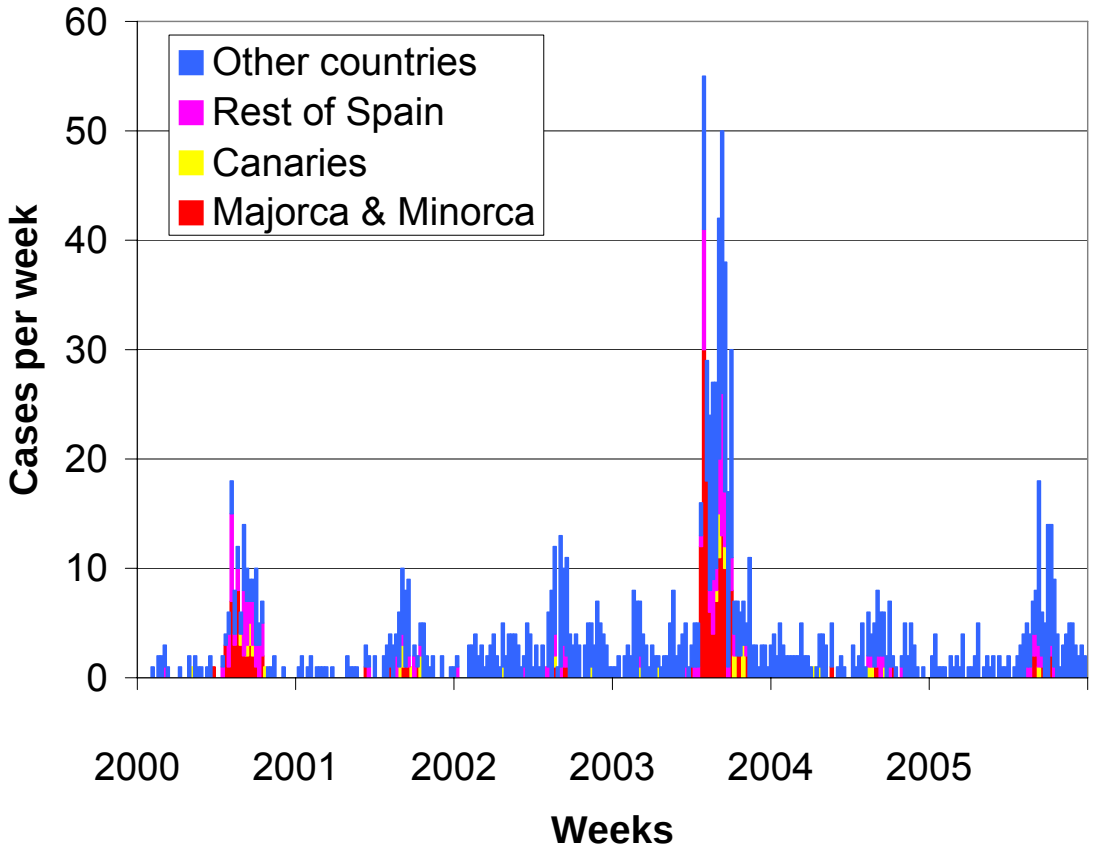
6.46 The majority of travel related cases occur at the same time as the early autumn general increase in Cryptosporidium cases (Figure 21). Because travel related cases are not ascertained in an effective way it is likely that travel cases are under-represented relative to the total but it is likely that travel plays an important role in the summer/autumn peak in cases.

Figure 21. Seasonal distribution of travel related disease



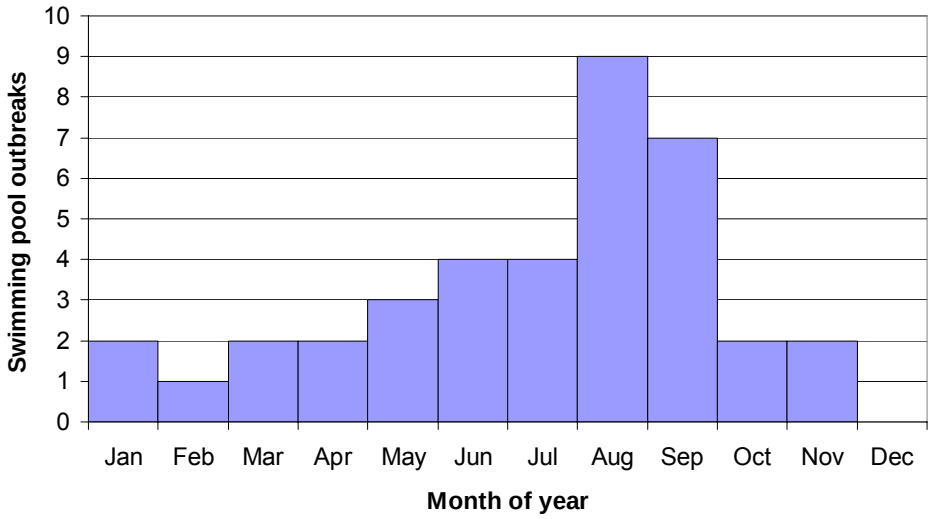
6.47 There have been two large outbreaks in 2000 and 2004 that have probably been caused by exposure of UK residents to hotel swimming pools in Majorca (Figure 22). As with all travel data, complete information on such outbreaks is difficult to collect because cases are dispersed across the country.

Figure 22. Cryptosporidiosis cases in England and Wales related to travel to Spain and other countries



6.48 Swimming pool outbreaks appear to be more common in the early autumn period (Figure 23). It is possible that this distribution reflects the greater numbers of people participating in swimming either at home or abroad during this period and is linked to the greater occurrence in travellers at this time.

Figure 23. Seasonal distribution of swimming pool outbreaks (England & Wales



6.49 **Comparison of the distribution of *C. parvum* and *C. hominis*.**

6.50 Data from between January 2000 and December 2003 show that *C. parvum* and *C. hominis* cases were similar with regard to gender (Table 5) but that the mean age of *C. parvum* cases was younger than *C. hominis.* However, this belied the distribution where *C. hominis* was more prevalent in 15-44 year olds, particularly females (Figure 24). *C. hominis* was also more prevalent than *C. parvum* in all cases under 1 year of age. In the case-control study of sporadic cryptosporidiosis *C. parvum* was more common in people under 20 years and *C. hominis* was more common it people aged 20 to 40 years (Hunter et al., 2004)(Figure 25).

Table 5. Demographics and history of cases with *C. parvum* and *C. hominis* in England and Wales 2000 to 2003

Variable		*C. parvum* n=3564	*C. hominis* n=3817	Chi square	p value
Age	Mean	15 years	17 years	9.69	0.002
	Range	0 to 92 years	0 to 97 years	-	
	Median; mode	8; 1 years	9; 1 years	-	
Gender	Female	1819 (51%)	1931 (50%)	1.00	
	Male	1733 (49%)	1866 (49%)	0.99	0.76
	Unknown	12 (<1%)	20 (1%)	1.54	0.21
Foreign travel	Yes	304 (9%)	621 (16%)	1.00	
	No	3260 (91%)	3196 (84%)	103.06	<0.001
Outbreak	Yes	276 (8%)	226 (6%)	1.00	
	No	3288 (92%)	3591 (94%)	9.66	0.002
Cluster	Yes	223 (6%)	331 (9%)	1.00	
	No	3334 (94%)	3478 (91%)	15.49	<0.001

Figure 24. Age and sex of 7381 cases with *Cryptosporidium* parvum and *Cryptosporidium* hominis between 2000 and 2003

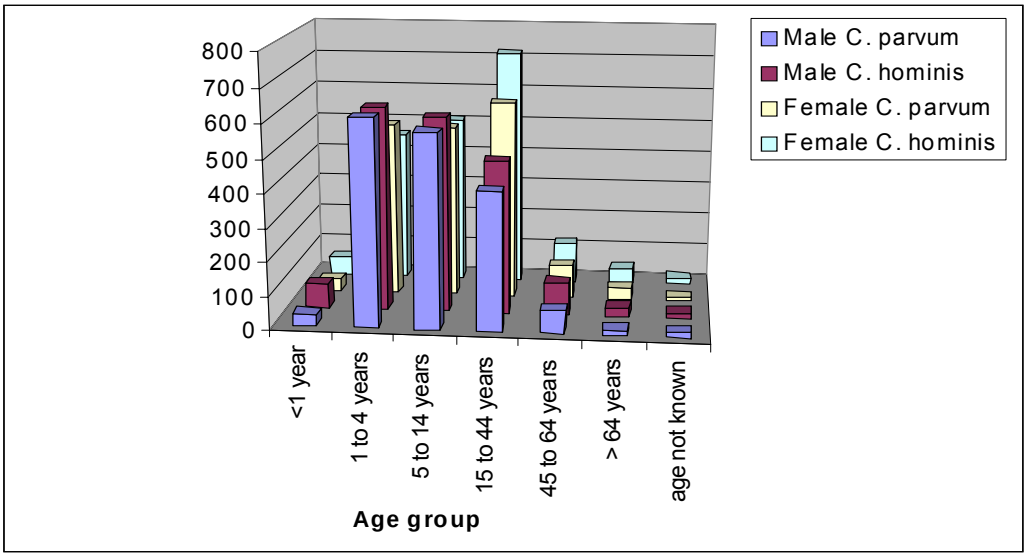
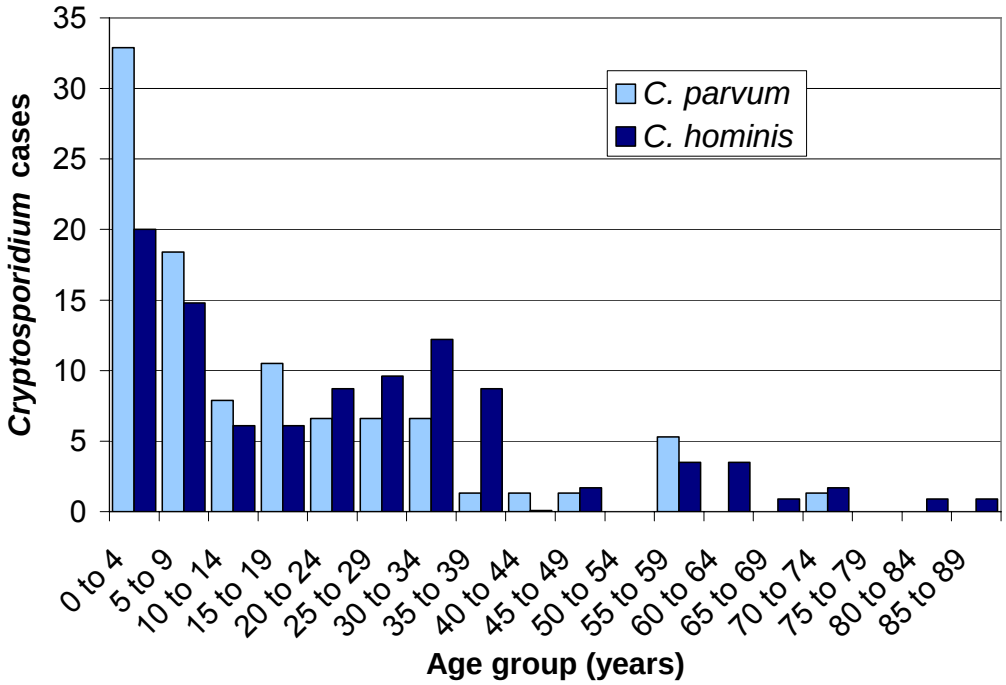


Figure 25. Age distribution of C. parvum and C. hominis in the case-control study of sporadic cryptosporidiosis (Hunter et al., 2004).



6.51 In recognised outbreaks there were more patients with *C. parvum* between 2000 and 2004, but in 2005 *C. hominis* predominated (Table 5; 6). By contrast fewer C. parvum cases belonged to family or household clusters than *C. hominis* cases (Table 6).

Table 6. *Cryptosporidium* species identified in outbreaks of cryptosporidiosis diagnosed in laboratories in England and Wales January 2000 to December 2005

CDSC ref. no.	Health Authority Region	Year	Month	Vehicle / route	Cases ill (lab. confirmed)	Faecal specimens typed by CRU	*Cryptosporidium* sp.
00/219	North West	2000	March	Public water supply	58 (58)	48	47 *C. parvum* 1 not determined
00/413	North West	2000	May	Public water supply	207 (207)	134	14 *C. hominis* 119 *C. parvum* 1 not determined
00/406	Trent	2000	May/ June	Public swimming pool	41 (41)	34	All *C. parvum*
00/440	South West	2000	May/ June	Farm holiday center / Private Water Supply	8 (3)	3	All *C. parvum*
~	London	2000	July/ Aug	Club swimming pool	9?	7	6 *C. hominis* 1 *C. parvum*
~	Mallorca	2000	Summer	Hotel swimming pool	>250	48	48 *C. hominis*
00/723	London	2000	July / August	Public swimming pool	5 (5)	1	*C. hominis*
00/656	London	2000	Sept	Public swimming pool	10 (10)	8	All *C. hominis*
00/870	South West	2000	Sept	Public swimming pool	12 (7)	1	*C. parvum*
00/806	London	2000	Oct	Day care Nursery	13 (13)	13	All *C. hominis*
00/972	South West	2000	Oct/ Nov	Club swimming pool	5 (5)	5	4 *C. hominis* 1 *C. parvum*
01/347	South East	2001	June	School swimming pool	152* (10)	5	All *C. hominis*
01/440	South West	2001	August	Environmental contact	14 (5)	5	3 *C. parvum*, 2 *C. hominis*
01/442	South East	2001	Sept	Day care Nursery	30 (10)	8	All *C. hominis*
01/528	South West	2001	Oct/ Nov	Club swimming pool	3 (3)	3	All *C. hominis*
02/018	North West	2002	March	College Private Water Supply	50** (1)	1	*C. parvum*
~	Wales	2002	May	Private Water Supply/farm/person to person	4 (4)	4	All *C. parvum*
02/1794	Northern and Yorkshire	2002	Nov	Day Care Nursery	47 (12)	9	8 *C. hominis* 1 not determined
02/1547	South East	2002	Nov	Public water supply suspected	21 (21)	18	All *C. hominis*
02/1701	South East	2002	Nov/Dec	Public water supply	31 (31)	28	All *C. hominis*
03/121	South East	2003	Feb	Swimming pool	20 (20)	5	4 *C. hominis* 11 *C. parvum*
03/167	Eastern	2003	March	Open farm, general public	6 (6)	2	Both *C. parvum*
03/197	Wales	2003	March	Open farm, school visit	17 (6)	6	All *C. parvum*
02/220	Northern and Yorkshire	2003	Jan-April	Swimming pool	66 (48)	21	All *C. hominis*
~	Wales	2003	April	Residential farm, school visit	36 (12)	10	All *C. parvum*
~	Mallorca	2003	July	Hotel swimming pool	?	16	14 *C. parvum* 2 not determined
03/411	West Midlands	2003	August	Interactive water feature	122 (35)	32	31 *C. hominis* 1 *C. meleagridis*

03/409	Waverley	2003	August/Sept	?swimming pools	17 (17)	2	Both *C. hominis*
03/401	South West	2003	September	Interactive water feature at an open farm	63 (31)	31	29 *C. parvum* 2 *C. hominis*
~	North West	2004	March	Swimming pool	4 (4)	3	*C. hominis*
~	Trent	2004	May	Open farm	9 (8)	8	*C. parvum*
04/186	N&Y	2004	May/June	Swimming pool	7 (7)	4	3x *C. hominis*
04/241	South West	2004	July	Open farm	20 (9)	7	*C. parvum*
~	Greece	2004	Sept	Wedding party	>20 (6)	3	*C. parvum*
04/371	N&Y	2004	Oct	Swimming pool	10 (9)	9	*C. hominis*
04/371	Wales	2004	Nov	Residential farm	3 (2)	2	*C. parvum*
05/076	South West	2005	Jan	Open farm	2 (2)	2	*C. parvum*
05/208	Wales	2005	Jan	Activity centre	2 (2)	2	*C. parvum*
~	North East	2005	April	Open farm	8 (8)	5	*C. parvum*
05/409	South West	2005	May	Campsite	2 (2)	1	*C. parvum*
~	Turkey	2005	Aug/Sept	Swimming pool (hotel)	?	2 identified	*C. hominis*
05/552	South East	2005	Sept/Nov	Public water supply	140 (140)	48	*C. hominis*
05/548	South East	2005	Sept/Nov	?	28	28	*C. hominis*
05/554	South East	2005	Sept/Oct	?	42	TBC	*C. hominis*
05/574	Wales	2005	Sept/Oct	?	TBC	TBC	*C. hominis*
05/574	London	2005	Aug/Dec	Swimming pools	84	TBC	*C. hominis*
~	Wales	2005	Oct/Dec	Public water supply	232	230	*C. hominis*

*concurrent community outbreak of NLV may account for a proportion of cases.

**outbreak of diarrhoea and vomiting. 1 *Cryptosporidium* case and 1 *Campylobacter* case confirmed.

PWS = Private water supply

6.52 Fewer *C. parvum* cases reported a recent history of foreign travel than *C. hominis* cases (Table 5). However, the distribution of species varied according to the continent visited, and patients returning from Asia and the Far East had a higher prevalence of *C. parvum* than *C. hominis*, although the numbers are too small to draw any general conclusion. These differences may reflect variations in the endemic *Cryptosporidium* genotypes in different countries (about which little is known) or differences in behaviour and exposure during travel to different destinations.

6.53 The distribution of cases has changed over time, both annually and seasonally with a decline in the number of *C. parvum* cases in each year since 2000 (Figure 26; 27). *C. parvum* was more prevalent in the spring to early summer and *C. hominis* was more prevalent in the late summer and autumn. The late summer / autumn peak includes more patients who had reported foreign travel whereas the spring peak in the *C. parvum* bovine genotype was almost exclusively composed of indigenous cases

6.54 Geographical differences were observed in the 2000 to 2003 dataset with *C. parvum* cases predominating in the North West, the South West and Wales and *C. hominis* predominating in Eastern, London, Northern and Yorkshire, South East and Trent. However, this distribution is not stable with a decline in cases of C.parvum in the North West being attributed to new water filtration (Sopwith et al., 2005).

Figure 26. Monthly variation of C. parvum and C. hominis by foreign travel reports, 2000 to 2003

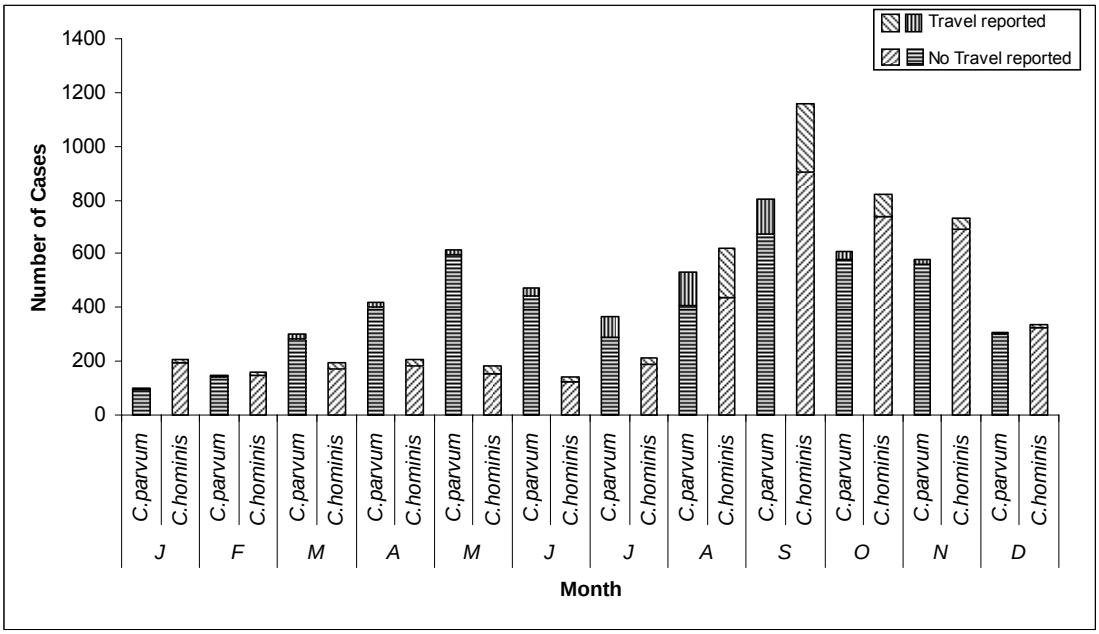
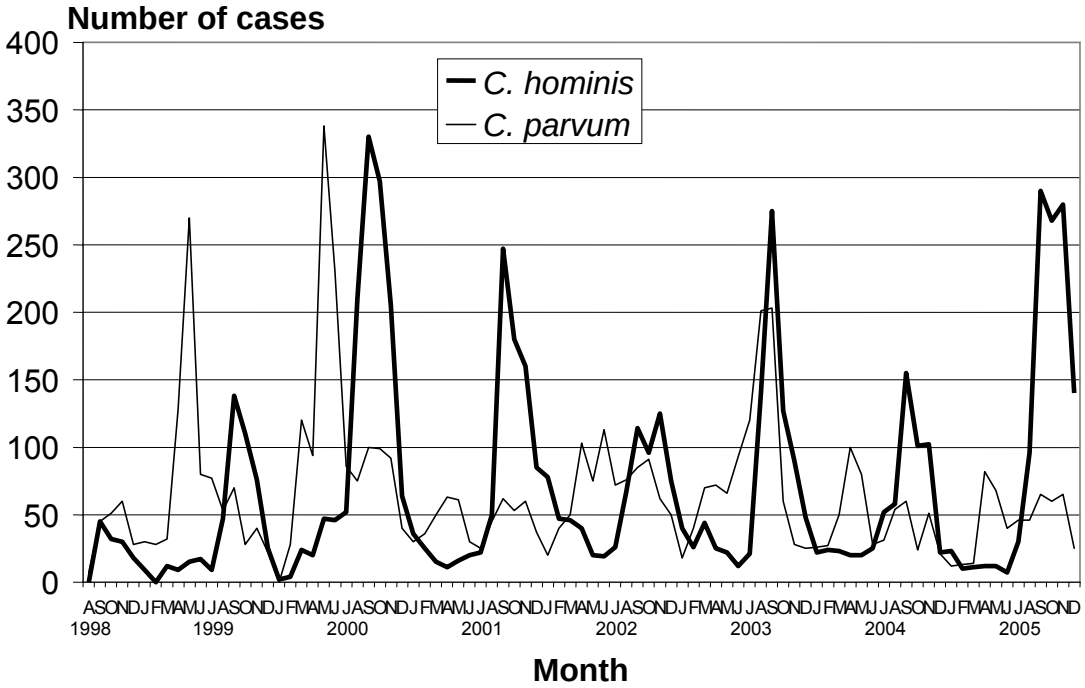


Figure 27. Annual monthly distribution of *C. parvum* and *C. hominis* over six years from 1989 to 2005



6.55 *Cryptosporidium* outbreaks.

6.56 Since 1983 there have been 149 detected outbreaks with a total of 9891 reported cases (Table 7; Appendices 11-14). The most common risk factor overall was the consumption of tap water unsurprisingly since the potential number of people exposed by this route, if the water supply is contaminated, is very large. There have also been many outbreaks associated with swimming pools, each of which has affected a smaller number of people. Although harder to detect because of their small size, outbreaks have occurred in private water supplies. Person to person spread (e.g. in nursery schools) and animal contact also give rise to small outbreaks or clusters of disease.

Table 7. Summary of *Cryptosporidium* outbreaks in the UK by transmission route: 1983-2005

Outbreak source/ transmission route	Total no. outbreaks	Total no. cases (lab positive)
Public drinking water supply	55	7097 (5821)
Private drinking water supply	6	176 (30)
Swimming pool	43	799 (490)
Interactive water features	3	191 (66)
Paddling pools	2	13 (6)
Other recreational water	2	27 (12)
Animal contact	16	936 (294)
Farm (transmission route unknown)	3	25 (19)
Food borne	4	140 (81)
Person-to-person	10	276 (111)
Unknown	5	148 (141)
Total	149	9851 (7071)

7 Risk factors

7.1 Drinking water

7.2 Cryptosporidiosis was first associated with a drinking water outbreak in 1983 (Appendix 14), although the epidemiological evidence was limited. The first published outbreaks were linked to a sewage contaminated well supplying a community in Texas (D'Antonio et al., 1985) and a large outbreak linked to a contaminated surface water source in Georgia, USA (Hayes et al., 1989). The first published outbreaks linked to drinking water occured in the England in 1986 (Rush et al., 1990) and in Scotland in 1988 (Smith et al., 1989; Smith et al., 1988).

7.3 For outbreaks in the UK where drinking water has been suspected as the most probable source the quality of evidence obtained has been variable. The evidence from outbreaks has been assessed using a published algorithm (Tillett et al., 1998). Outbreaks are classified as being associated with water either 'strongly', 'probably' or 'possibly'. This is a relatively crude device for categorising outbreaks and there is always some doubt about whether an outbreak was really caused by drinking water especially in the absence of a well executed case control study.

7.4 The large outbreak linked to drinking water in Oxford/Swindon in 1989 led to the establishment of an Expert Group under the chairmanship of Sir John Badenoch and subsequently by Professor Ian Bouchier. The Expert Group's work was directed mainly at identifying water supply mangement risk factors and how to prevent outbreaks due to public water supplies. The government introduced additional regulations to assess and mitigate the risk due to public water supplies in 1999.

7.5 Drinking water outbreaks only account for around 8% of disease and the role of public water supplies in the majority of cases is unclear. However, a strong correlation between rainfall and non-travel related cases of cryptosporidiosis in spring time, implies that many of these cases could have been related to water supplies without sufficiently robust water treatment (Lake et al., 2005).

7.6 Private water supplies

7.7 Private water supplies are commonly cited in rural locations and contamination of the waters is related to the security of the source, safe transmission from source to tap and whether any treatment is used.

7.8 There have been six *Cryptosporidium* outbreaks associated with private water supplies in England and Wales (Duke et al., 1996) (Appendix 15). Most of the outbreaks were associated with water that was in close proximity to agricultural animals. *Cryptosporidium* outbreaks in private water supplies are comparatively uncommon and *Campylobacter* infections are the commonest cause of outbreaks linked to private water supplies (Said et al., 2003).

7.9 Outbreaks associated with private water supplies are likely to be poorly detected due to difficulties in detecting small outbreaks in communities with a private supply.

7.10 Bottled water

7.11 Bottled water can occasionally become contaminated with *Cryptosporidium* oocysts, particularly in developing countries (Franco and Cantusio, 2002) where water safety is suboptimal. The consumption of municipal water compared to bottled water was a risk factor in an outbreak in Mexico (Leach et al., 2000). In outbreak investigations where mains drinking water is the likely source of infection bottled water consumption may come out of case control studies as protective, because people have drunk this instead of mains water. Demonstrating an outbreak linked to such a contamination event is likely to be difficult in the absence of national sub-typing of *Cryptosporidium* isolates from patients.

7.12 Ice

7.13 An outbreak was identified as being caused by ice from an ice machine in a hospital ward, and contamination was by direct contact with the ice by an incontinent, psychotic patient with cryptosporidiosis (Ravn et al., 1991). While ice may represent a risk factor in similar situations there is experimental evidence that oocysts are rendered less viable by freezing, although a risk may still be present.

7.14 Swimming pools

7.15 A number of outbreaks of cryptosporidiosis have been associated with swimming pools (Mathieu et al., 2004; Lim et al., 2004; Louie et al., 2004; Puech et al., 2001; 2001a; 2001b; Stafford et al., 2000; Sundkvist et al., 1997; Lemmon et al., 1996; Mackenzie et al., 1995; Wilberschied, 1995; McAnulty et al., 1994; 1994; Hunt et al., 1994; Bell et al., 1993; Sorvillo et al., 1992; Joce et al., 1991; Insulander et al., 2005). Outbreaks linked to swimming pools in England and Wales were reviewed in 2000 (Anon, 2000).The main contributing factors identified have included sewage contamination of pool water through a broken drain (Joce et al., 1991), faecal accident (Hunt et al., 1994), defective filtration and backwashing during pool use rather than at the end of a day. Some outbreaks were in pools where ozone treatment had been discontinued. For the many outbreaks there was no identifiable practice or feature that was linked to human disease.

7.16 Interactive water features

7.17 Outbreaks of cryptosporidiosis have been linked to interactive water features (Nichols, 2006; Jones et al., 2006). These are situated outside and are combinations of water jets and pools. The treatment of such water is often sub-optimal and contamination from the environment probably occurs frequently. These features are

not always designed with adequate filtration to accommodate the greater contamination of water they experience.

7.18 Other recreational water

7.19 Outbreaks have been associated with a recreational lake in a state park in New Jersey (Kramer et al., 1998) and a recreational water park in Illinois (Causer et al., 2006). In the UK outbreaks there have been two outbreaks linked to paddling pools, one to recreational use of a river, one to contamination of a stream on a beach. Recreational waters involved in these outbreaks have been ones that would not be covered under the EC Bathing Water Directive (European Union, 2006).

7.20 Direct animal contact

7.21 Cryptosporidiosis can occur as a result of direct contact with animals and infections have been reported in veterinary workers (Preiser et al., 2003; Reif et al., 1989; Pohjola et al., 1986), in people exposed to animals (Stantic-Pavlinic et al., 2003) or amongst those dealing with agricultural animals as part of their job, particularly in developing countries (Mahdi and Ali, 2002; Rahman et al., 1985).

7.22 Farm visits

7.23 Where children attend farms as a residential camp or single day educational or recreational activity there is a risk of being infected by zoonotic infections including *C. parvum* (Smith et al., 2004; Stefanogiannis et al., 2001; Sayers et al., 1996; Evans and Gardner, 1996; Dawson et al., 1995; Elwin et al., 2001). Such premises need to ensure appropriate washing facilities for children and adults (Dawson et al., 1995; Pritchard et al., 2006) although infections can still occur even with these precautions (Evans and Gardner, 1996). The HSE has published guidance for those operating open farms. www.hse.gov.uk/pubns/ais23.pdf

7.24 Pets

7.25 *Cryptosporidium* can be detected in both cats and dogs (Lallo and Bondan, 2006; Fontanarrosa et al., 2006; Ederli et al., 2005; Huber et al., 2005; Cirak and Bauer, 2004; McGlade et al., 2003; Hackett and Lappin, 2003; Abe et al., 2002; Mtambo et al., 1991) and the species present can include *C. felis*, *C. canis*, *C. parvum* and *C. meleagridis* (Zhou et al., 2004; Hajdusek et al., 2004). Although human infection in the UK with *C. felis*, *C. canis*, and *C. meleagridis* is uncommon, people have been infected with these species (Leoni et al., 2003; Pedraza-Diaz et al., 2001b; Pedraza-Diaz et al., 2001a; Chalmers et al., 2002c). Pet food can be contaminated with enteric pathogens, including *Cryptosporidium* (Strohmeyer et al., 2006), and this may contribute to the infections in animals. A case control study, funded by Defra, is currently underway to estimate and evaluate risks of cryptosporidiosis from pets.

7.26 Fairs and shows

7.27 An outbreak has been associated with an animal nursery in a fair in Tasmania (Ashbolt et al., 2003). An outbreak at a fair in the US was linked to apple cider (unfermented apple juice) consumed at the fair (Millard et al., 1994). An outbreak was associated with an event in Minnesota where food was thought to be the source (Anon, 1996a).

7.28 Visits to the countryside

7.29 The large outbreak of Foot and Mouth Disease in the UK in 2001 led to a dramatic reduction in access to the countryside. In this year an equally dramatic reduction in cases of cryptosporidiosis in many parts of the UK was observed (Goh et al., 2005; Strachan et al., 2003; Hunter et al., 2003; Smerdon et al., 2003). Some of this reduction will have been due to reduced direct animal to human contact by the general population (Smerdon et al., 2003) and the remainder has been attributed to new water filtration (described earlier).

7.30 Family spread

7.31 Family outbreaks have been recognised for many years (Ribeiro and Palmer, 1986), but these are not routinely recorded in outbreak surveillance data. In all community outbreaks irrespective of the source there will be secondary transmission within families. The national scheme for *Cryptosporidium* typing has shown that cases involved in family clusters were more likely to have *C. hominis* than *C. parvum* (Table 5)

7.32 Nurseries and schools

7.33 *Cryptosporidium* can be easily transmitted from person to person within nurseries, day care settings and schools (Hannah and Riordan, 1988; Cruickshank et al., 1988). Sporadic transmission between children in these settings is probably fairly common while outbreaks associated with childcare are relatively uncommon.

7.34 Hospital

7.35 Cryptosporidiosis can be transmitted within the hospital environment (el Sibaei et al., 2003; Squier et al., 2000; Craven et al., 1996; Gardner, 1994; Ravn et al., 1991) and can be a significant problem on units that deal with immuno-compromised patients such as bone marrow transplant units (Martino et al., 1988).

7.36 **Camping**

7.37 Camping activities can be responsible for outbreaks of diarrhoeal diseases through the consumption of contaminated water, inadequate food hygiene, contact with animals or bathing in contaminated water . Such outbreaks can be caused by multiple pathogens (Smith et al., 2004) as well as *Cryptosporidium* alone (Anon, 1996b).

7.38 **Sexual activity**

7.39 Infection with *Cryptosporidium* in HIV positive people was associated with men who have sex with men (MSM) (homosexual men) more commonly than IV drug users, suggesting sexual transmission (Pedersen et al., 1996). A small study conducted in Australia amongst MSM identified sexual behaviour as an important risk factor for infection with *Cryptosporidium* (Hellard et al., 2003). Men having more than one sexual partner per month, engaging in anal penetrative sex and attending a sex venue were all at increased risk of infection. HIV positive people can get more severe illness associated with *Cryptosporidium* infection (Kim et al., 1998; Jougla et al., 1996; Lopez-Velez et al., 1995) and prevention has focussed on drinking bottled or boiled water. However, high risk behaviours remain common in this group (Kim et al., 1998).

7.40 **Food and drink**

7.41 *Cryptosporidium* is one of a number of parasitic protozoa that can be transmitted through food (Nichols, 2000; Dawson, 2005; Dawson et al., 2004; Duffy and Moriarty, 2003; Rose and Slifko, 1999). Detection of these outbreaks can be difficult due to technical difficulties in detecting the oocysts in food (Laberge et al., 1996) and lack of routine typing of isolates (Caccio and Pozio, 2001). A number of groups have examined methods for detecting oocysts in foods (Kniel and Jenkins, 2005; Cook, 2003; Robertson and Gjerde, 2001a; Deng and Cliver, 2000; Deng et al., 2000; Bier, 1991). Other work has examined the effects of various food treatments on oocysts (Dawson et al., 2004; Dawson et al., 2004; Kniel et al., 2003). Raw ingredients may be contaminated as may the water used for food processing, particularly in developing countries (Sutthikornchai et al., 2005) but also from irrigation water in developed ones (Thurston-Enriquez et al., 2002). *Cryptosporidium* oocysts are sensitive to hot water and it has been suggested that this can be a suitable way of cleaning and decontaminating carcases (Moriarty et al., 2005). Foodborne outbreaks of cryptosporidiosis may occur in tourist resorts where they are unlikely to be detected through normal surveillance processes (Cartwright, 2003).

7.42 **Unpasteurised milk**

7.43 Dairy product related outbreaks have been reported in the UK (Djuretic et al., 1997; Gelletlie et al., 1997) but have only occurred where the milk was improperly pasteurised.

7.44 **Fruit juice**

An outbreak in the US was linked to apple cider (unfermented apple juice) consumed at the fair (Millard et al., 1994). The source of contamination may be the washing process (Garcia et al., 2006) or animal faeces within orchards. UV irradiation has been suggested as a treatment for these products (Hanes et al., 2002).

7.45 **Raw salads**

7.46 Raw salad items including sprouted beans may be a source of infection through contaminated water during production and processing (Robertson et al., 2005; Robertson and Gjerde, 2001b)

7.47 **Flies**

7.48 Flies have been postulated as important vectors for the transmission of *Cryptosporidium* oocysts (Hiepe and Buchwalder, 1991). Experimental work on *Musca domestica* (The house fly) suggests that the organism can be transmitted (Graczyk et al., 2000; Graczyk et al., 1999b; Graczyk et al., 1999a) and oocysts have been recovered from flies in a cattle barn and around a landfill site (Szostakowska et al., 2004). There is no epidemiological evidence for fly transmission of cryptosporidiosis, although the epidemiology of fly transmission is difficult (Nichols, 2005).

7.49 **Molluscs**

7.50 Molluscs filter large volumes of water that is commonly contaminated with animal and human faecal waste. Such molluscs have been postulated as a route of transmission of *Cryptosporidium* (Graczyk and Schwab, 2000). *Cryptosporidium* has been detected in mussels and oysters (Li et al., 2006; MacRae et al., 2005; Chalmers et al., 1997b). A variety of processing approaches to remove *Cryptosporidium* contamination have been tried (Collins et al., 2005a; Collins et al., 2005b)

7.51 **Transmission by food-handlers within kitchens**

7.52 An outbreak of cryptosporidiosis in a cafeteria in Washington DC was associated with a food-handler (Quiroz et al., 2000).

7.53 **Travel abroad**

7.54 Cryptosporidiosis has long been recognised as being associated with travel abroad (Black, 1986; Sterling et al., 1986; Jokipii et al., 1985; Soave and Ma, 1985). Because the distribution of cryptosporidiosis is worldwide it is commonly found in people returning from other countries.

7.55 Travel related cases have been more commonly found to be caused by *C. hominis* than by *C. parvum* (McLauchlin et al., 2000) (and Table 5). The implication that much of the infection is derived from infected people rather than animals. Travel related cases have been more commonly found in the late summer months (McLauchlin et al., 2000)(and Table 5). Travel abroad has been identified as an independent risk factor for *Cryptosporidium* infections in the US (Khalakdina et al., 2003; Roy et al., 2004) and for *C. hominis* infection but not *C. parvum* infection in England and Wales (Hunter et al., 2004). While water and food quality are thought to play an important role (Cartwright, 2003), there is an absence of good analytical evidence of the risk factors that are responsible for transmission within holiday resorts.

7.56 There have been outbreaks associated with hotel complexes in Majorca where swimming pool water and filters were contaminated with oocysts. When large outbreaks occur in foreign resorts they can be detected as an increase in cases throughout England and Wales. Because surveillance is not particularly good in some countries serological examination has been used to assess differences in exposure in different countries (Frost et al., 2000).

7.57 Cryptosporidiosis in England and Wales has been associated with travel to Spain in general and Majorca in particular (Table 8). There have been large outbreaks of cryptosporidiosis linked to hotel swimming pools in Majorca in 2000 (C. hominis) and 2003 (C. parvum) (Table 8). Between 1 January 2000 and 31 December 2002, 219 laboratory confirmed cases of cryptosporidiosis in English and Welsh holidaymakers returning from Majorca were reported, including an outbreak associated with a pool in Calles de Mallorca during 2000 (CDR 11 August 2000; CDR 7 Aug 2003).

Table 8. Countries associated with travel related cryptosporidiosis cases in England and Wales 1999-2005

Resort country3	1999	2000	2001	2002	2003	2004	2005
Africa	4	12	15	15	16	9	13
Indian subcontinent	16	13	20	17	20	25	22
Spain, Portugal and islands	12	82	21	16	159	17	17
The rest of Europe	7	13	22	7	28	4	9
West Indies and Central America	1	5	3	3	10	7	
Middle East	1	2	2		6	3	1
Southern Asia	2	8	5	3	4	5	11
Far East and Australia	4	5	1	1	3	1	
North America	2	2	1		1	1	1
South America	1			1	2		1
Country Not known	2	3	6		2	1	3
No recent travel	5000	5688	3549	2974	5672	3540	4451
Grand Total	5052	5833	3645	3037	5923	3613	4529
Total travel associated	52	145	96	63	251	73	78
% from Spain	0.24	1.41	0.58	0.53	2.68	0.47	0.38

7.58 Travel away from home

7.59 The outbreak in Torbay in 1995 was associated with an increase in cases nationally and this is likely to have been as a result of holiday makers in the Torbay area becoming ill after returning home from holiday (Nichols, 2003).

7.60 Cruise ships

7.61 Although outbreaks of cryptosporidiosis linked to cruise ships are uncommon one has occurred (Rooney et al., 2004a; Rooney et al., 2004b). An outbreak of cryptosporidiosis occurred on an US Coast Guard Cutter that filled its tanks with water from Milwaukee during the period when there was a large waterborne outbreak (Moss et al., 1998).

7.62 **Routes of transmission of *C. parvum* and *C. hominis***

7.63 The routes of transmission for *C. parvum* and *C. hominis* contain some aspects that are similar and some that are different. *C. parvum* can occur commonly within agricultural animal populations whereas *C. hominis* does not). The prevalence of human cryptosporidiosis in England and Wales differs between these two species.(Figures 28 & 29) and the distribution of cases has changed in the last few years.

Figure 28. Transmission pathways for *C. parvum*

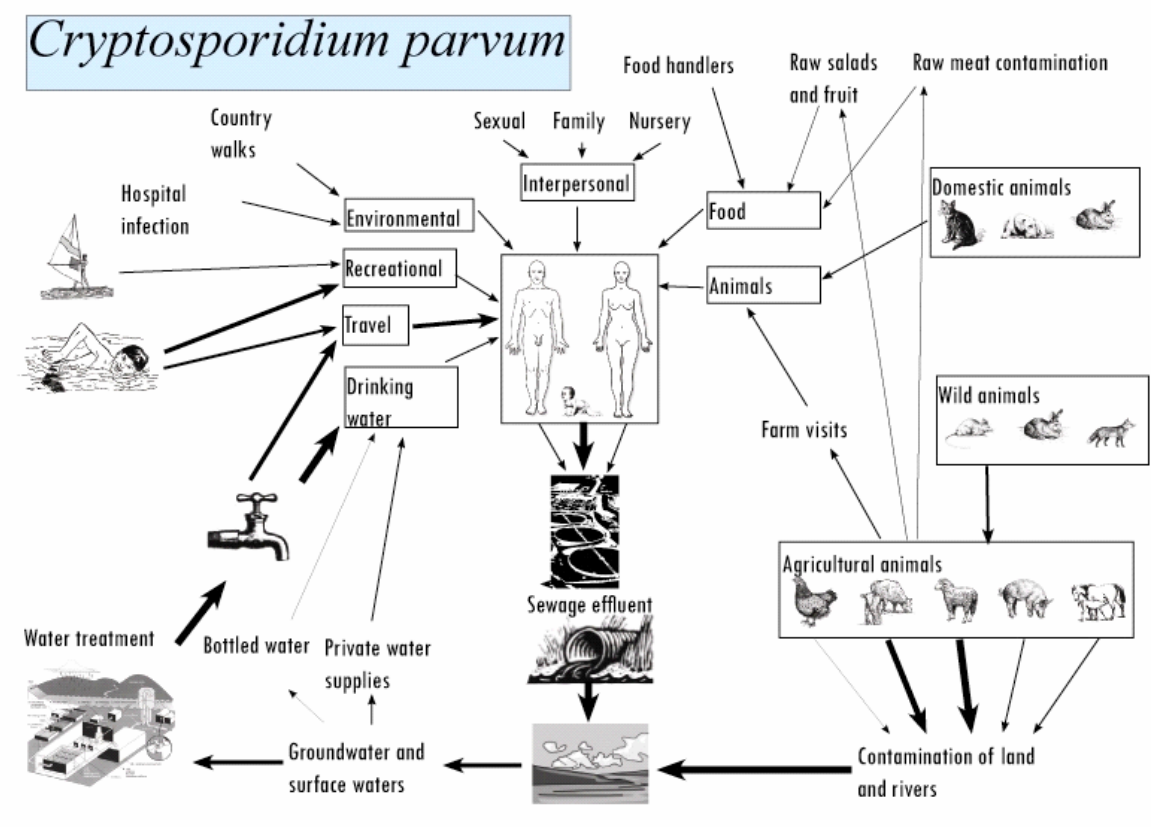
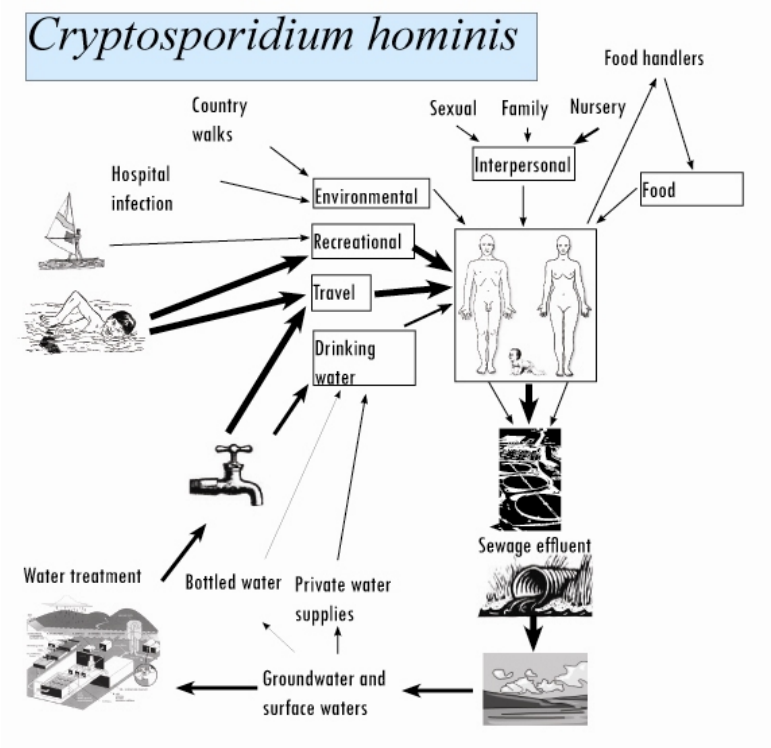


Figure 29. Transmission pathways for *C. hominis*



7.64 **Measuring the disease burden resulting from drinking water**

7.65 The burden of disease attributed to various sources remains unclear, however because drinking water treatment interventions were introduced in Cumbria and the North West with a consequential reduction in disease it may be possible to use these data to estimate how much disease is attributed to drinking water.

7.66 There is also some evidence for farmed animal and human host-adapted strains of *C. parvum* (Anon 2005;(Mallon et al., 2003b; Mallon et al., 2003a))

8 Drinking water and cryptosporidiosis

8.1 The three expert reports (Badenoch, 1990; Bouchier, 1998; Badenoch, 1995) identified a number of problems with contamination of drinking water supplies and their recommendations remain very relevant today. They have had major influences in how water companies design and operate their water treatment. Many of the pieces of evidence outlined below have been discussed in these reports.

8.2 Evidence of what causes waterborne cryptosporidiosis

8.3 In order to establish the causes of drinking water outbreaks and to prevent their repetition it is important that water providers understand the range of problems that can lead to outbreaks. This can be referred to as critical factor identification and control (CFIC). This approach has been used in the past to respond to outbreaks of waterborne disease. Following the Maidstone typhoid outbreak in 1897-8 there was the introduction of chlorination. The typhoid outbreak of 1937 in Croydon highlighted the need to chlorinate after repairs in a well. There are many other more recent examples and many have been included in a recent review (Hrudy and Hrudy, 2004). The Critical Factors associated with *Cryptosporidium* outbreaks are outlined below.

8.4 Backwash re-cycling.

8.5 Evidence from the *Cryptosporidium* outbreak in Oxford and Swindon suggested that re-cycling backwash water to the head of the works was a practice that can amplify the treatment challenge (Richardson et al., 1991). This process of water conservation is no longer used routinely at surface water works.

8.6 Filtration bypass.

8.7 There is evidence from an outbreak in Hull that bypassing individual filters at a treatment works in response to a difficulty with supplying sufficient water to the community was a contributory factor.

8.8 Slow sand filters

8.9 Slow sand filtration is generally effective at removing *Cryptosporidium* oocysts. Nonetheless outbreaks have been associated with water supplies where slow sand filtration is used (Northern Ireland and Hull) and operational deficiencies were identified.

8.10 Performance of filtration

8.11 Filters differ in their efficiency at removing oocysts. A number of outbreaks appear to have occurred partly as a result of oocysts passing through the filters (Hayes et al., 1989). Filters vary in their efficiency to remove oocysts through the cycle of their use and the periods immediately before and after backwashing have been highlighted as posing a greater risk of breakthrough. Likewise sub optimal clarification and flocculation processes have been suggested as a factor (Hayes et al., 1989).

8.12 Animal contamination of source waters

8.13 In a number of outbreaks the contamination of drinking water has been attributed to contamination with faecal matter from agricultural animals. However, it is easy to make assumptions regarding the source of the contamination and retrospective application of typing methods has shown that some of these outbreaks may have been due to human as opposed to agricultural sources of water source contamination (Chalmers et al., 2002a), highlighting the importance of typing during such investigations.

8.14 Sewage contamination of source waters

8.15 A number of outbreaks have been associated with the contamination of surface waters with sewage. There has also been an outbreak where sewage appears to have contaminated groundwater (Willocks et al., 1998). There have also been clusters of outbreaks where there appears to have been a link between drinking water and sewage (N London, Herts & Beds outbreaks - elaborate).

8.16 Impact of drought on groundwater vulnerability

8.17 Drought can lead to a lowering of the water table that can result in changes in underground water flows, and incursion of surface water into groundwater that is not normally so affected. There have been a number of *Cryptosporidium* outbreaks where beforehand there has been a dry period followed by a period of rain (Willocks et al., 1998; Bridgman et al., 1995).

8.18 Impact of drought on effluent dilution

8.19 Long dry periods can lead to less dilution of sewage effluent and animal wastes in surface water-courses with the result that river sources may become more contaminated with oocysts. This may have been a contributory factor in at least one outbreak where the intrusion of river water into groundwater was a hypothesised transmission route (Willocks et al., 1998).

8.20 Heavy rainfall

8.21 Waterborne outbreaks of cryptosporidiosis have been associated with episodes of heavy rainfall and flooding (Bridgman et al., 1995). A study of 548 US outbreaks of waterborne disease found fifty-one percent were preceded by precipitation events above the 90th percentile (P = .002), and 68% by events above the 80th percentile (P = .001) (Curriero et al., 2001). For surface water related outbreaks the closest associations were seen with rainfall in the preceeding month and for groundwater related outbreaks, rainfall two months beforehand. In a study of all cases of cryptosporidiosis in England and Wales associations were found between run-off events (caused by heavy rainfall) and cryptosporidiosis especially in the spring (Lake et al., 2005).

8.22 Oocyst concentrations have been found to be higher in samples after rainfall compared to other sampling periods (Hansen and Ongerth, 1991). The contamination of surface waters with oocysts after heavy rainfall is likely to result in short lived peaks of contamination.

8.23 Treatment operation

8.24 Inadequacies in the operation of drinking water production have been identified in the majority of drinking-water-borne *Cryptosporidium* outbreaks in England and Wales. Outbreaks are predominantly as a result of breakdowns in water treatment rather than the effects of rainfall, climate, river water quality etc. In point 3.1.5 of the Bouchier report it states that “The Group concluded from its examination of these incidents that outbreaks of water related cryptosporidiosis do not just happen. There appears to be a strong correlation between outbreaks and situations where an inadequacy was identified in the treatment provided or in the operation of the treatment process. ….”(Bouchier, 1998).

8.25 Contamination of groundwater

8.26 Groundwater contamination has been responsible for many outbreaks (Lee et al., 2002; Barwick et al., 2000; Moore et al., 1993) including outbreaks of cryptosporidiosis within England and Wales (Willocks et al., 1998; Bridgman et al., 1995). Although the mechanism for the transmission of contamination from surface waters to groundwater was identified in one outbreak (Bridgman et al., 1995) in another the source was less clear (Willocks et al., 1998). Both these outbreaks were linked to heavy rainfall prior to the outbreak. In one of the outbreaks the source was found to a surface water drain leading directly from a field containing livestock faeces.(Bridgman et al., 1995).

8.27 Cross contamination through plumbing

8.28 An outbreak in Northern Ireland was associated with the ingress of wastewater from a blocked drain (Glaberman et al., 2002).

8.29 Backflow and cross connection

8.30 In most distribution systems the possibilities for backflow along pipes is reduced through the use of non-return valves. An outbreak of waterborne disease was associated with pressure differences in a rural supply causing untreated source water to pass into distribution (Gutteridge and Haworth, 1994).

8.31 Post treatment contamination

8.32 An outbreak in Scotland was associated with irregular seepage of oocyst-containing water, which increased during heavy rains, into a break-pressure tank containing final water for distribution, rather than a failure of the water-treatment processes (Smith et al., 1989).

8.33 Network repair

8.34 The infectious risks associated with repair work on water utility infrastructure have been well understood since the Croydon outbreak of typhoid in 1937. Following this there hygiene protocols were introduced to mitigate against this risk. Poor hygiene procedures have been implicated in at least one protozoan outbreak (Jephcott et al., 1986).

8.35 Problems in aqueduct integrity

8.36 An outbreak in Northern Ireland was associated with ingress of sewage into an aqueduct (Glaberman et al., 2002).

8.37 Outbreak clusters

8.38 There have been occasions where two or more outbreaks occur that appear to be temporally linked but with different sources of infection. This was seen in North London in 1997 when during a large public water supply outbreak (Willocks et al., 1998) was at the same time as other outbreaks in adjacent counties and linked by a river . There have also been smaller community outbreaks linked to several swimming pools (Puech et al., 2001).

8.39 Repeated outbreaks

8.40 There have been occasions where an outbreak has recurred in the same place in a subsequent year. This can happen with drinking water, swimming pool or animal farm related outbreaks. This suggests that the main contributing factors were not identified and acted upon following the first outbreak or that the interventions

were insufficient to mitigate the risk. This may have been the case in two outbreaks in Torbay in 1992 and 1995 (Appendix 14).

8.41 Streaming and lake water

8.42 The Oxford/Swindon outbreak in 1989 provided the first evidence that storage of surface water in a natural lake or manmade reservoir may not serve to reduce the risk of raw water contamination. *Cryptosporidium* oocysts do not settle out quickly and mixing in water bodies is a complex process that varies seasonally and with weather. Streaming was also thought to be a problem in the North West outbreak associated with a

8.43 Ice melting

8.44 Outbreaks or waterborne disease have followed the blocking of sewage as a result of large amounts of snow and ice melting (Anderson, 2001; Millson et al., 2006) although no *Cryptosporidium* related outbreaks have been reported. .

8.45 Turbidity control

8.46 Turbidity has been seen to be increased in some *Cryptosporidium* outbreaks (Badenoch, 1990) and control of turbidity is used as an additional measure for reducing the risk of *Cryptosporidium* oocyst contamination of drinking water. During the large waterborne outbreak in Milwaukee the drinking water had an increased turbidity (Mac Kenzie et al., 1994), and strong associations between turbidity and gastroenteritis-related emergency room visits and hospitalizations occurred at temporal lags of 5-6 days (consistent with the Cryptosporidium incubation period) (Naumova et al., 2003).

8.47 Some studies have shown no clear cut relationships between oocyst contamination and turbidity (Sacco et al., 2006) while others have shown removal of oocysts and turbidity to be correlated (Hsu and Yeh, 2003). Evidence suggests that water without any turbidity can still be contaminated with oocysts, but removing turbidity tend to will remove oocysts. Particle counting can give better associations with *Cryptosporidium* oocysts (Brookes et al., 2005). Turbidity can also influence oocyst recovery rates (DiGiorgio et al., 2002), with turbid waters giving poorer recovery than non-turbid ones.

8.48 Protective foods

8.49 Some criteria have been associated with a reduced risk of cryptosporidiosis in case-control studies during outbreaks and in studies of sporadic disease. Eating ice-cream, and raw vegetables (particularly tomatoes) was protective (Hunter et al., 2004). The reason for the protection is not fully understood. It could be a direct inhibitory effect of these products on excysted sporozoites within the small intestine, but there may be other explanations, including aspects of study design, bias and

confounding. There is little evidence of a mechanism to explain these observations and it is doubtful whether consuming these products regularly within a diet would confer any real protection.

8.50 The role of immunity

8.51 Following an infection with *Cryptosporidium* the parasite is normally eliminated from the body within a couple of weeks through an immunological process. The immunity that results from infection confers a degree of protection against subsequent re-infection. Human volunteer experiments carried out in Texas showed that volunteers could be infected with an ID50 of 132 oocysts (Chappell et al., 1996; DuPont et al., 1995). Subsequent re-infection of these patients a year later identified lower oocyst shedding and although similar numbers of patients were symptomatic the symptoms were less severe (Okhuysen et al., 1998). The ID50 of defined strains of *C. parvum* differ (Messner et al., 2001; Okhuysen et al., 1999). There are similarly differences in host response to *C. hominis* compared to *C. parvum*.

8.52 From these and other studies it is possible to draw some conclusions about exposure and immunity.

1. Infection causes partial immunity.
2. People being re-infected may be ill without shedding oocysts in numbers capable of detection by faecal specimen screening.
3. Regular exposure is likely to cause regular infection but with reduced disease

8.53 Big mistakes

8.54 A recent fault tree analysis has identified a number of mistakes that have contributed to the occurrence of outbreaks around the world (Hunter, in press).

1. Not learning from our or others big mistakes.
2. Thinking that because you know of no outbreaks in your country everything must be OK, even if you have not looked.
3. Thinking that Ground water is safe and that it does not require any more treatment just because it has come out of the ground.
4. Thinking that Surface water is safe just because you have chlorinated it.
5. Stopping worrying about water safety after it has left the water works. Assuming that the people who built the water works years ago knew all about *Cryptosporidium*.
6. Thinking that cows and sheep grazing near the water works gives a more attractive rural setting.
7. Assuming everything works the same after heavy rainfall.
8. If source water is pristine there is no need to install more expensive treatment.

9. Under-estimating the ingenuity of man to contaminate clean drinking water after it has left the water works.
10. Assuming that your people know when laboratory results are indicating a problem and what to do about it.
11. Forgetting to thank the people who have done most of the work.

8.55 **Swimming pools**

8.56 From the examination of swimming pool outbreaks it is possible to determine a number of factors that play a contributory part. There is no evidence that contaminated drinking water used as a water source within pools has ever contributed to outbreaks.

8.57 There is little evidence that contamination of indoor pools is from animal sources because there is no access to animals in these premises. Infection is thought to derive from the faeces of infected people, especially young children. One outbreak has been attributed to leakage of toilet drains into pool water (Joce et al., 1991).

8.58 Faecal accidents by children are surprisingly common and may be unrecognised by pool staff. Although there is only circumstantial evidence that outbreaks are linked to faecal accidents and little evidence evidence that outbreaks could have been prevented through following these following the correct procedures for dealing with these is good practice.

8.59 There has been circumstantial evidence of poor filtration efficiency as a result of low pool turnover and poor filter maintenance. How much these contribute to outbreaks is unclear, and there are no good tests of filter efficiency that can be applied in an outbreak to test how well fliters are performing.

8.60 Some of the outbreaks may have been associated with backwashing. Unlike drinking water filtration, swimming pools have re-circulating water that should be further cleaned with each pass through the filter. Backwashing can temporarily disrupt filter integrity. It is recommended that this should be undertaken at the end of a day when the pool water can be recirculated back through the filters after any breakthrough resulting from the backwash has happened. More than one outbreak has occurred at a time when backwashing was conducted in the middle of the day.

8.61 A number of outbreaks have occurred in pools where ozone treatment was used but had been discontinued. It is unlikely that the move away from ozone was a direct cause of these outbreaks and this may represent a reflection of disruputed pool management practices.

8.62 High bather density may contribute to outbreaks (Causer et al., 2006). Large swimming pools are potentially a greater risk because more people are likely to contaminate the pool and more people will be exposed. However, large pools should have bigger and potentially more efficient filtration systems to remove oocyst contamination.

8.63 Many of the outbreaks have involved infants and children in learner pools. These pools are smaller and separate circulation systems that can be drained down when contamination occurs. Where separate filtration is not in place there can be a risk to people using other pools that have the same circulation.

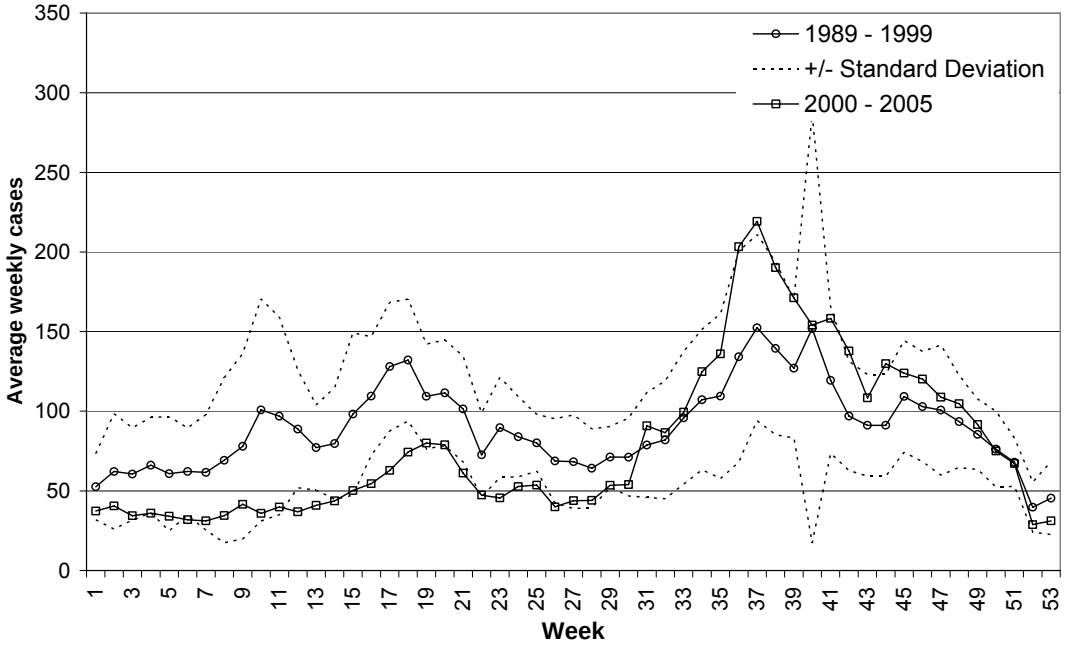
8.64 *Cryptosporidium* oocysts and *Giardia* cysts can be detected in swimming pools in the absence of cases of cryptosporidiosis or giardiasis (Bonadonna et al., 2004; Greinert et al., 2004; Schets et al., 2004). The relatively common detection of *Cryptosporidium* (11.8%), *Giardia* (5.9%) or both (1.3%) in five pools in the Netherlands does suggest that exposure may be common through swimming (Schets et al., 2004). In a case-control study in New South Wales there were more commonly detected in pools where two or more cases had swum (Puech et al., 2001).

8.65 Naked pre-swim showering has been suggested as a way of reducing the occurrence of faecal matter (and *Cryptosporidium* oocysts) on skin in the anal area. Evidence that this will reduce outbreaks is not available but it is recommended as good practice.

9 Impact of improvements in the water supply

9.1 The purpose of this section is to examine the changes in cryptosporidiosis that may have occurred since *The Water Supply (Water Quality) (Amendment) Regulations 1999* (commonly referred to as the *Cryptosporidium* Regulations) were introduced. A reduction in *Cryptosporidium* levels in drinking water has been suggested (Lloyd and Drury, 2002) and since the regulations a reduction in reported cases of cryptosporidiosis, especially the disappearance of the spring peak, has been observed in the North West (Sopwith et al., 2005). This is indicated in Figure 30, which compares the mean weekly cryptosporidiosis cases in England and Wales 2000 - 2005 with 1989 - 1999. The numbers in the first half of the year have been lower after the regulations (2000 – 2005). Although the *Cryptosporidium* Regulations became law in 1999 the treatment improvements were frequently not in place until 2-3 year later.

Figure 31. England and Wales average weekly Cryptosporidiosis cases 1989 – 1999 and 2000 – 2005 (cases reporting recent travel cases excluded)



9.2 In Figure 30 the standard deviation lines are also presented which give an indication of how much the mean numbers of cases each week have differed from year to year in the period 1989 - 1999. They indicate a large year-to-year fluctuation in weekly incidence before the regulations. This makes it difficult to ascertain whether the post 2000 decrease represents a real reduction in cryptosporidiosis cases or is part of the natural variability in incidence. It also makes it problematic to quantify the numbers of cryptosporidiosis cases, if any, avoided by the new regulations.

9.3 **Analysis Overview**

9.4 The purpose of this analysis is to model the causes of the year-to-year fluctuation in weekly incidence in the period 1989 – 1999 (before the new regulations). As discussed in previous sections it is likely that some of this inter annual variability is due to weather and community spread from infected people returning from abroad. Once these causes have been modelled the resulting relationships can be used to predict the numbers of cases that would have been expected between 2000 and 2005 had the new regulations not been implemented. These predictions can then be compared to the actual numbers of cases observed allowing us to examine the impact, if any, of the new regulations. This allows us to examine whether the changes in incidence post 2000 are statistically significant, and to quantify any associated public health benefit.

9.5 The analysis builds upon previous research (Lake et al., 2005), and develops models to predict reported non-travel cryptosporidiosis cases in the whole of England and Wales using incidence data reported to national surveillance between 1989 and 1999. The predictions are based upon weekly temperature, rainfall, river flow and travel cases. The development of the predictive models is described in Appendix 13.

9.6 In these models it was important to ensure that weeks with unusually high or low numbers of cryptosporidiosis cases were not having a large influence on the results. This was achieved by fitting the models with and without such data. The results demonstrated our models to be robust and not driven by a few unusual weeks such as those during the Torbay or North London outbreaks.

9.7 **Post Regulation 2000 – 2005**

9.8 The models presented in Appendix 13 were used to predict the non-travel cryptosporidiosis cases that would have been expected to occur in each week between 2000 and 2005. We have also estimated the 95% confidence interval of each estimate. The results are presented in Figure 31-36. In these graphs the observed weekly cryptosporidiosis cases are plotted alongside the predictions and their associated upper and lower 95% confidence intervals. If there is a consistent pattern of observed cases being different to those predicted then this provides some confidence that the observation is not a chance occurrence. If any one week falls outside the 95% confidence limits then there is only a 5% probability that the result for that week is a chance occurrence.

9.9 These Figures demonstrate a consistent pattern of weekly cryptosporidiosis cases in the first half of the year being below the predictions. In many weeks, especially during the spring, the numbers are also less than the 95% confidence interval. This provides strong evidence of a reduction in cryptosporidiosis in the first half of the year since 2000.

9.10 In order to test this statistically Table 9 was created which combines the data from individual weeks into a single value and confidence interval for the first half of

the year. This demonstrates that in the first half of the year the observed numbers of cases are significantly lower than the model predictions.

9.11 The impact is most striking in 2001 when the observed cases are consistently below the 95% confidence interval of the predictions between weeks 10 and 34. This coincides with the 2001 Foot and Mouth disease epidemic that led to the slaughter of over 6 million livestock and severely restricted public access to agricultural land.

Figure 31: England and Wales predicted and observed cryptosporidiosis 2000 (cases reporting recent travel cases excluded)

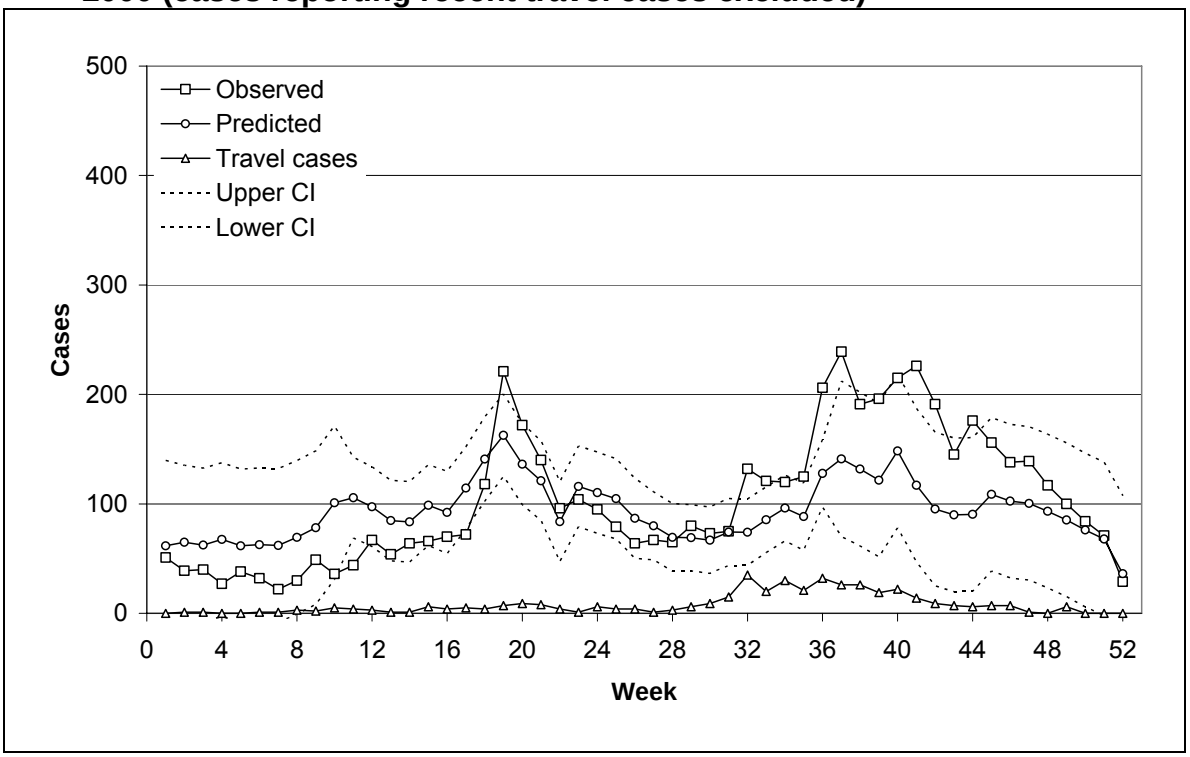


Figure 32: England and Wales predicted and observed cryptosporidiosis 2001 (cases reporting recent travel cases excluded)

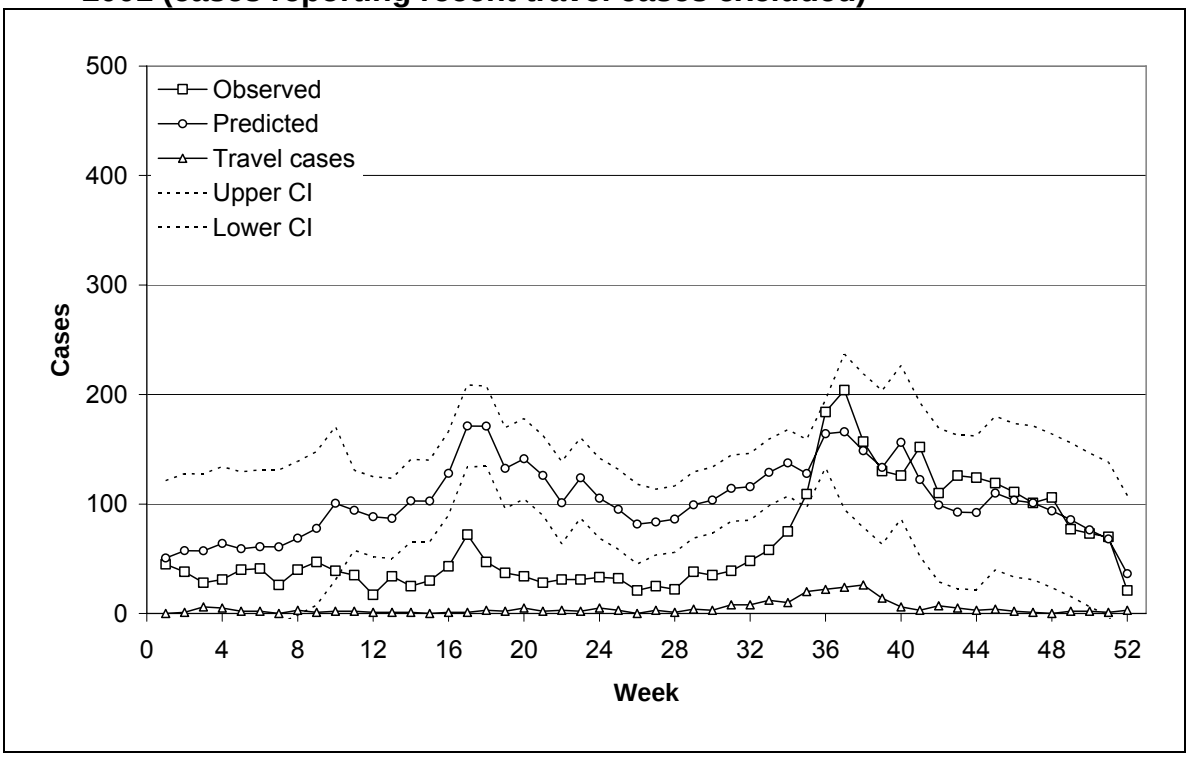


Figure 33: England and Wales predicted and observed cryptosporidiosis 2002 (cases reporting recent travel cases excluded)

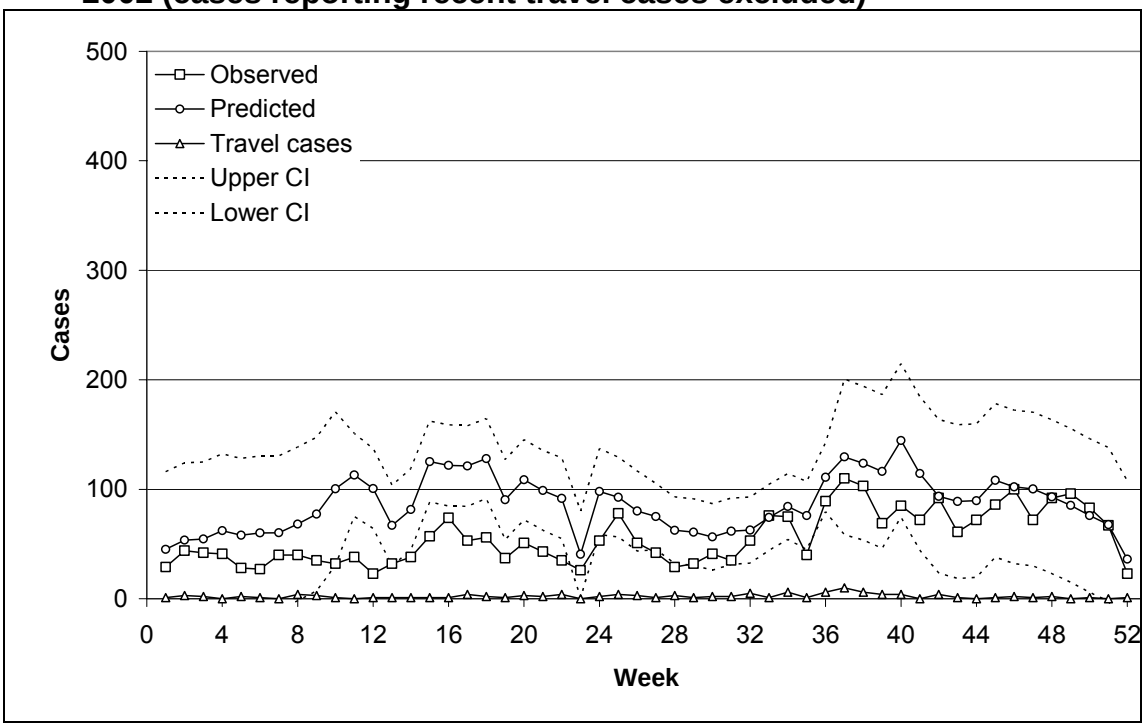


Figure 34: England and Wales predicted and observed cryptosporidiosis 2003 (cases reporting recent travel cases excluded)

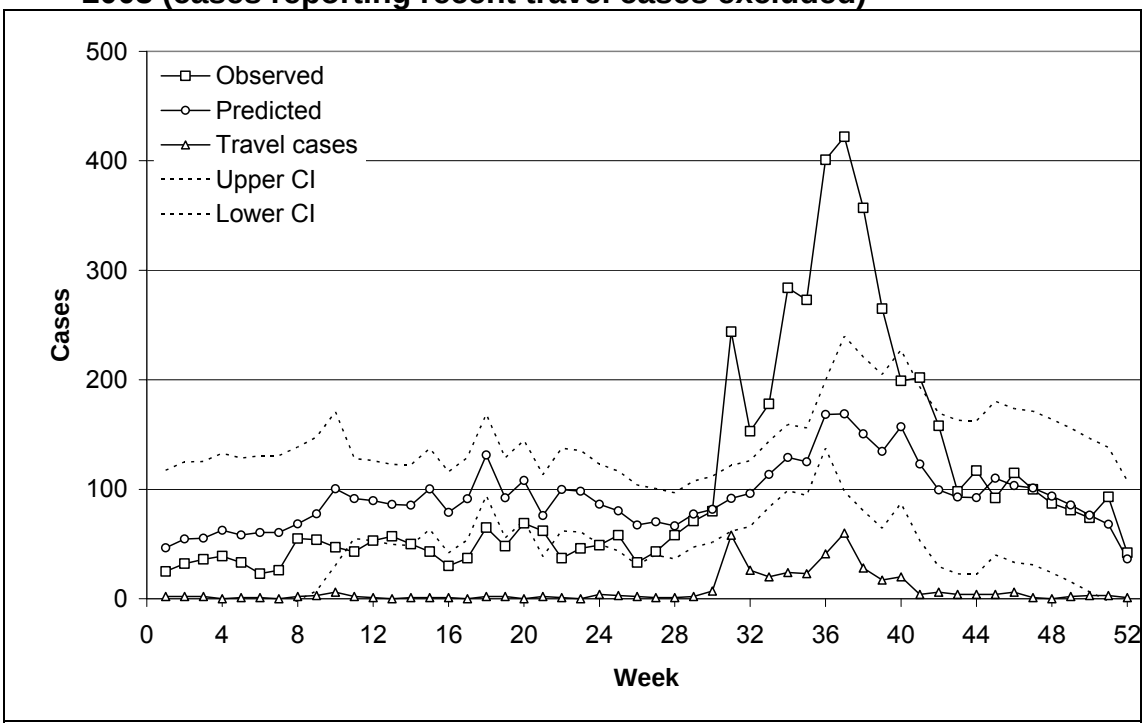


Figure 35: England and Wales predicted and observed cryptosporidiosis 2004 (cases reporting recent travel cases excluded)

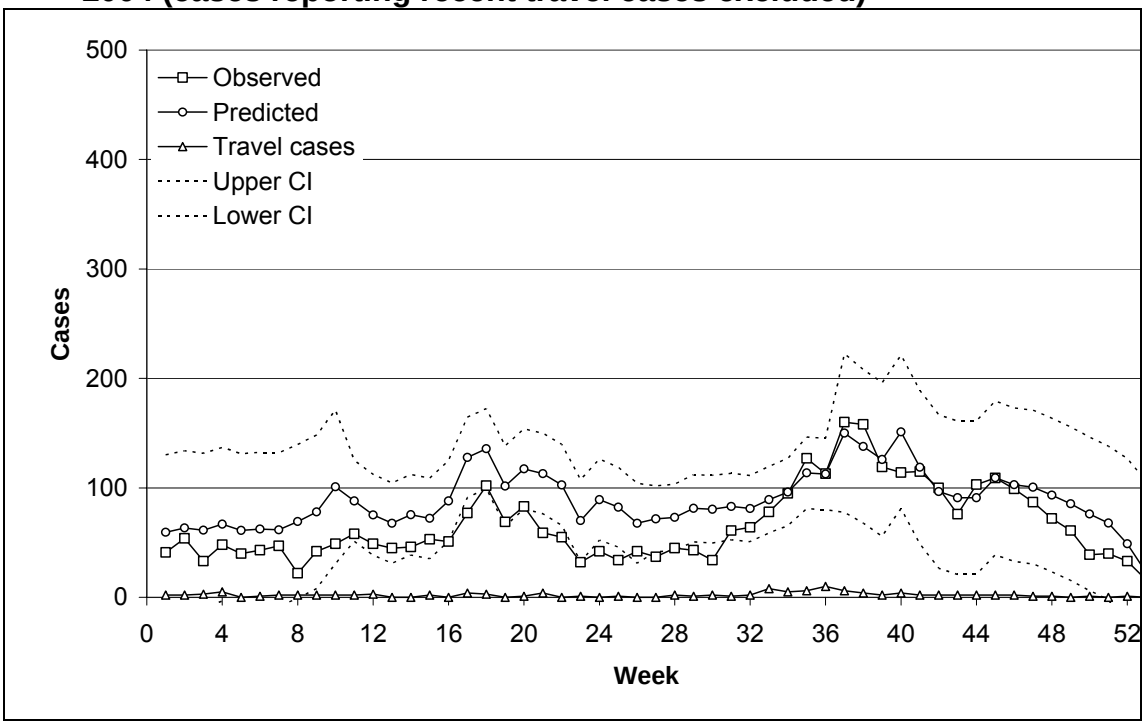
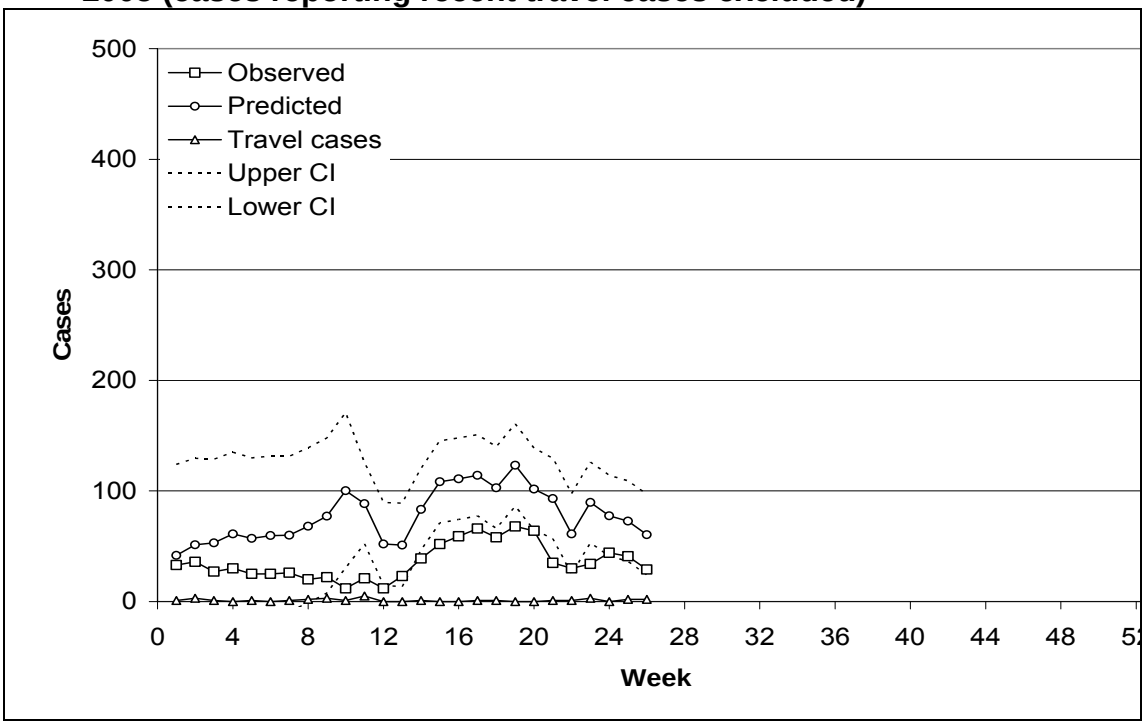


Figure 36: England and Wales predicted and observed cryptosporidiosis 2005 (cases reporting recent travel cases excluded)



9.12 In addition to drinking water improvements it is important to consider whether there are any other plausible explanations for the decline in cryptosporidiosis post 2000. One possibility could be lower *Cryptosporidium* incidence in livestock following the Foot and Mouth epidemic in 2001 (Hunter et al., 2003; Smerdon et al., 2003) as the incidence in re-stocked livestock may initially have been lower (Strachan et al.,

2003). However, a recent study has demonstrated high rates of cryptosporidiosis in rodents living around farm buildings and highlights these as an important factor of re-infection in livestock (Sturdee et al., 2003). It is therefore likely that any decreased incidence in livestock would not persist for many years (Sopwith et al., 2005). Furthermore, any decreased incidence in livestock due to Foot and Mouth would not explain the reductions in cases observed in 2000.

9.13 If we turn our attention to the second half of the year then the pattern post 2000 is less straightforward. In 2001, 2002 and 2004 (Figures 32, 33 and 35) the numbers of cases accord well with the model predictions. However, in 2000 and 2003 (Figure 31 and 34) the numbers of cases are consistently and sometimes significantly above the model predictions. One explanation for the excess cases in the second half of 2000 and 2003 are that they represent unreported travel cases or community transmission as a consequence of people returning home from holidays with the infection. Reported travel cases are plotted on Figure 31-36 and an association between total cases and foreign travel is apparent. However, foreign travel was not a significant explanatory variable of weekly cryptosporidiosis cases 1989 - 1999. This anomaly may be because in 2000 and 2003 the number of reported travel cases was at a higher level than at any time between 1989 and 1999. It may also be due to improved recording of travel cases in recent years.

9.14 It is also worth noting that the autumn peak is dominated by the *C. hominis* genotype. There are no natural non-human reservoir hosts for *C. hominis* and so most illness is likely to be related to foreign travel, person to person transmission or contamination of drinking water by human sewage (McLauchlin et al., 2000).

9.15 These results are examined statistically in Table 9. This demonstrates that for the second half of the year, and for every year, the observed numbers of cases are significantly different to the model predictions. However, in 2000 and 2003 the results are significantly higher and in 2001, 2002 and 2004 the results are significantly lower. This inconsistency, combined with the potential link to travel cases and the predominance of the *C. hominis* genotype lead us to conclude that the changes seen in cryptosporidiosis post 2000 in the second half of the year are less likely to be related to drinking water supplies. It remains possible that the increases in disease in the autumn, which may be resulting from a combination of foreign travel and community transmission, are masking changes in the levels of disease related to drinking water. It should be noted that because of reporting delay , data for the latter half of 2005 has not be included in the analysis.

9.16 By comparing observed to predicted cryptosporidiosis cases an estimate of the public health benefits of the new drinking water regulations can be produced. These are presented in Table 10. In this analysis we have omitted data for 2001 because of the confounding effect of foot and mouth. If we compare over the whole time period then there were around 615 less cases of cryptosporidiosis reported each year. This equates to a reduction in incidence from 8.9 per 100000 to 7.7 per 100000. Data from a study of infectious intestinal diseases (IID) estimated that only 1 in every 7.4 cases of cryptosporidiosis in 1995 appeared in national surveillance (Adak et al., 2002) implying that around 4550 cases have been avoided each year. Such multipliers are relatively crude and probably change over time. Recent re-analysis of the IID study samples using molecular methods indicates that using

conventional microscopy alone laboratories are missing about the same number of cases as they are detecting.

9.17 However, this number masks a large decrease in the first half of the year, offset by a small increase in the second half (Table 10). We argue that the increase in cases in the second half of the year is less likely to be associated with drinking water, although there is little analytical data to support this. Therefore, a more liberal estimate of cases avoided would focus upon the first half of the year only resulting in an annual reduction of 905 reported or 6700 total cases. This implies that the new drinking water regulations have had most impact in reducing infection with *C. parvum* as this species dominates in the first half of the year.

Table 9: Observed and predicted cryptosporidiosis England and Wales 2000, 2002, 2003, 2004 and 2005

Time Period		Observed Cases	Predicted Cases	95% CI	99% CI	p	Summary
First half of year (weeks 1-26)	2000	1890	2431	2162 - 2700	2076 - 2785	<0.05	Decrease
	2001	925	2510	2243 - 2776	1892 - 3128	<0.01	Decrease
	2002	1103	2200	1933 - 2467	1848 - 2551	<0.01	Decrease
	2003	1150	2107	1840 - 2374	1756 - 2458	<0.01	Decrease
	2004	1316	2159	1892 - 2425	1808 - 2509	<0.01	Decrease
	2005	931	2021	1744 - 2297	1656 - 2385	<0.01	Decrease
Second half of year (weeks 27-52)	2000	3477	2438	2140 - 2735	1748 - 3126	<0.01	Increase
	2001	2461	2927	2627 - 3226	2233 – 3620	<0.05	Decrease
	2002	1795	2294	1997 - 2591	1605 - 2983	<0.05	Decrease
	2003	4287	2713	2416 - 3010	2025 - 3401	<0.01	Increase
	2004	2198	2552	2241 - 2863	1831 -3272	<0.05	Decrease

Table 10: Estimated changes in cryptosporidiosis England and Wales 2000 – June 2005[1]

Time Period	Changes in reported cases		Changes in total cases[2]	
	2000 – June 2005	per annum	2000 – June 2005	per annum
Whole year	-2767	-615	-20478	-4550
January to June	-4527	-905	-33501	-6700
July to December	1760	440	13024	3256

[1] The projections exclude 2001 due to the potential confounding effect of the foot and mouth epidemic
[2] Calculated by assuming that the ratio of total cases to reported cases is 7.4

9.18 Conclusions about disease changes in recent years

9.19 In summary this research presents strong evidence that the annual reductions in cryptosporidiosis in England and Wales post 2000 are not due to the natural variability in cases but can be attributed to the new drinking water regulations. We estimate, based upon the modelling, that the Regulations have saved 615 reported cases per annum (an estimated 4500 cases in total). A larger decline has been seen in the first half of the year which is offset by a small increase in cases in the second half of the year. When the reductions were examined geographically there were

indications that the overall improvement in the situation was concentrated in the west of the country but also there was some affect in the South East. Conversely there were indications that cryptosporidiosis rates in the east of the country, where the overall incidence is lower, were on the increase.

9.20 The effect of the Foot and Mouth outbreak on the reduction in cryptosporidiosis has been difficult to disentangle from the impact of improved drinking water treatment. Using the modelling in this study we estimate a decrease in cases due to FMD activity of about 850, representing over 6300 community cases.

9.21 These data provide strong evidence for widespread improvements in the quality of drinking water as measured by consumer illness since 2000. The reduction in the spring cases has not completely eradicated the seasonal effect suggesting there may still be some drinking water related illness. The autumn increase in cases is largely unexamined by analytical epidemiological methods and warrants investigation.

9.22 Water quality

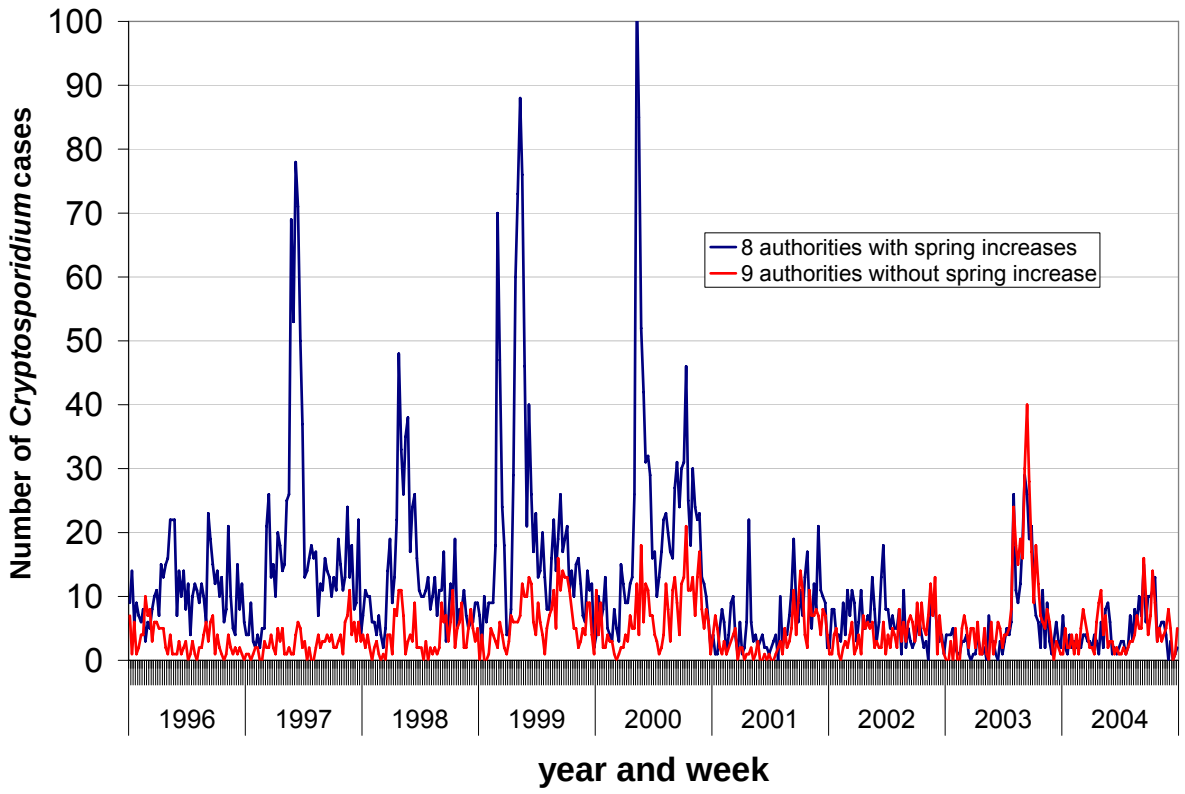
9.23 The extent to which mains drinking water is contaminated with *Cryptosporidium* oocysts has been clarified by the data from the *Cryptosporidium* monitoring of these supplies organised under the *Cryptosporidium* regulations 1999. These data are predominantly from water works assessed to be at significant risk. There is little data from other water supplies. To date there has been little examination of the relationship between water quality and disease with cryptosporidiosis in England and Wales. This is because the post-code location of affected patients was not recorded routinely in surveillance datasets. A methodology for conducting such an analysis has been published (Hall et al., 2002). The increased capture of post-code data through surveillance (see Figure 8) means that conducting such an epidemiological analysis is now feasible.

9.24 Changes in the North West

9.25 The reductions in the North West have been well documented (Sopwith et al., 2005). Because outbreaks in the North West Region have been prominent, a study examined local authorities affected by a large waterborne outbreak and plotted cases back in time to demonstrate previously unrecognised outbreaks (Hunter et al., 2001). The data from all North West local authorities has been re-plotted for the years 1996 to 2004 to illustrate how the improvements in water supply and treatment have impacted on the spring cases. There were 8 local authorities that had spring cases in more than one year between 1996 and 2000 (Bury & Rochdale, East Lancashire, Manchester, Morecambe Bay, North Cumbria, NW Lancashire, Salford & Trafford, Wigan & Bolton) and nine the did not (Liverpool, North Cheshire, Sefton, South Cheshire, South Lancashire, Saint Helens & Knowsley, Stockport, West Pennine, Wirral) (Figure 37). Most of the Health Authorities showing spring cases were ones almost entirely fed by the Thirlmere aqueduct whereas North Cumbria receives a different supply that was the subject of case-control and intervention studies (Goh et al., 2005; Goh et al., 2004) (this supply wasn't part of the NW region

at the time of the previous analysis). East Lancashire has a dynamic mix of supplies that are mostly not from Thirlmere. The various interventions can be seen to have impacted on cryptosporidiosis cases in both spring and autumn since 2001, with the two groups of authorities now having much more similar patterns of infection than between 1996 and 2000.

Figure 37. Cryptosporidiosis cases per week in the North West Region between 1996 and 2004



10 Guidance documents on cryptosporidiosis

10.1 Guidance on outbreak detection, investigation and management

10.2 Guidance on the detection, investigation and management is provided in the expert reports produced by under the Chairmanship of Badenoch and Bouchier. There is a need for this guidance to be refreshed and disemminated and there is probably a need for specific guidance about the investigation of outbreaks linked to swimming pools.

10.3 Guidance for immuno-compromised patients

10.4 Patients with impaired T-cell count and function are at increased risk of severe disease with cryptosporidiosis but the risk varies depending on the condition (Hunter and Nichols, 2002). Current CMOs advice, issued in 1999 is:

> *Cryptosporidium* in water: clarification of the advice to the immunocompromised
> "The Bouchier Report *Cryptosporidium* in Water Supplies: Third Report of the Group of Experts (1998)[1] included advice for the immunocompromised. This was publicised in the February 1999 edition of CMO's Update[2]. A working group of specialists chaired by Professor Ian Bouchier have now defined further which groups of immunocompromised patients are at particular risk of cryptosporidiosis infection and should boil their drinking water.
>
> The level of T-cell function and the duration of any immune suppression were considered to be crucial factors in susceptibility to *Cryptosporidium*. The group concluded that the advice should be that anyone whose T-cell function is compromised (this includes people with HIV infection who are immunosuppressed, children with severe combined immunodeficiency (SCID) and those with specific T-cell deficiencies, such as CD40 ligand deficiency, also known as Hyper IgM Syndrome, should be advised to boil and cool their drinking water from whatever source. This includes tap or bottled water, and ice cubes should also be produced from boiled and cooled water."

10.5 However, there is a need for the most vulnerable patient groups to be defined. For example, the introduction of new immunomodulating drugs for rheumatology patients has created a new group with significantly impaired T-cell function in whom an increase risk from tuberculosis has been identified. There would seem to be a need for medical advice to be updated in light of thse changes as it is unclear whether the CMOs advice should be extended to those taking highdose steroids, patients who have had bone marrow, stem cell or organ transplants and patients on anticancer chemotherapy resulting in depressed T cell function.

10.6 **Guidance on boil water notices and other interventions**

10.7 One of the areas of greatest difficulty in respect of the management of waterborne outbreaks is when and where to apply a boil-water notice. There are a number of interventions that can be used in a waterborne outbreak to reduce the risk of infection to the population served by drinking water source that may be contaminated. The boil water notice serves two main functions. The first is to warn the public that the drinking water is potentially contaminated and the second is advising them that boiling the water is an effective way of making the water safe to drink. When introduced the compliance with advice can be relatively high but reduces over time (Willocks et al., 2000). Some of the boil notices have lasted for an extended period (Wallis et al., 2001), and the criteria for stopping a boil water notice after it has been introduced have not been clearly worked out in advance (Harrison et al., 2002). Boil notices have an unfortunate impact on outbreak investigations in that they advertise to the public that drinking water is a potential source of illness. This is thought to result in recall bias and over reporting of water consumption in case-control studies, although there may be recall bias from press interest in the absence of a boil water notice (Hunter and Syed, 2002). There has been concern that scalding incidents are more common during boil water notices, although the evidence supporting this concern is limited. Supplying bottled water for drinking may be an option although there can be logistic issues if the geographical area is large. Boil notices can also cause a lack of trust in consumers.

10.8 Intervention is one of the fundamental tenets of public health protection. Where there are concerns that water supplies are acutely at risk, as during an outbreak, it is vital that risks are rapidly assessed and decisions made about whether there are any practical interventions, including a boil-water notice, that might reduce the risk of many more people becoming infected. Where there is a chronic problem with the contamination of drinking water the practicality of achieving long term compliance with a boil water notice significantly limits its potential for public health benefit.

10.9 Specific updated guidance on boil-notices and other interventions would be useful.

11 Conclusions and recomendations

11.1 The evidence from surveillance studies and epidemiological studies is that there has been an overall reduction in cryptosporidiosis associated with the introduction of the *Cryptosporidium* Regulations and consequential improvements in water treatment. This is especially evident in the first half of the year. This provides further evidence, on top of investigations of *Cryptosporidium* outbreaks and analytical studies of sporadic cryptosporidiosis, that drinking water has been responsible for a substantial burden of this diarrhoeal disease. It has provided an example of how new legislation can significantly impact beneficially on the burden of waterborne disease. However, there may still be some element of the burden of disease that is associated with drinking water and the epidemiological evidence is far from clear about the remaining causes of cases.

11.2 **Further investigation of the seasonal increase in cryptosporidiosis is needed to determine general and species-specific risk factors for acquisition and transmission of infection, and to enable appropriate interventions for prevention and control to be instituted. This is particularly true for the cases in the latter half of the year.**

11.3 Spring peak

11.4 The spring increase that was prominent nationally before 2000 has declined but is still evident, if much reduced.

11.5 **There is a need to determine whether the remaining spring cases are related to drinking water or some other source and an analytical study would be useful in determining this.**

11.6 The late summer/autumn increase

11.7 The drinking water regulations together with measures taken by water companies appear to have been responsible for causing the reduction in cryptosporidiosis in the first half of the year. These measures do not appear to have resulted in a similar reduction in the autumn. These results might be explained in a number of ways. It is possible that in the spring the majority of disease is drinking water related as a consequence of raw water contamination associated with periods of lambing and the release of animals on the land. Although the Cryptosporidium Regulations have been successful in reducing this risk some further refinement may be needed. In the second half of the year the drinking water risk is unlikely to be due to highly infectious young animals or run off from land. However at this time of the year access to the countryside is at its highest and so direct contact with animals

may be an important risk factor. Furthermore, in the late summer England and Wales usually experiences an influx of infections from abroad. Finally at this time the use of swimming pools is at its highest leading to local spread of cryptosporidiosis. However, the real source of *Cryptosporidium* cases in the second half of the year is unclear.

11.8 **An analytical study is required to examine the risk factors for cryptosporidiosis in the autumn.**

11.9 Risk status, oocyst contamination of drinking water and sporadic disease

11.10 A number of supplies produce water that occasionally contains low numbers of oocysts. The significance of these has always been questioned because the method of water testing measures oocysts which can be non-viable and of species that do not commonly infect humans. On top of this there is evidence that strains can differ in their ability to infect people (Teunis et al., 2002; Okhuysen et al., 1999). On the other hand with some strains having an ID50 of less than 10 (Okhuysen et al., 1999), and the ID50 for children possibly being lower than this (human volunteer studies cannot be conducted on children), it remains possible that low counts of oocysts represent some risk of infection. Furthermore, spatial heterogeneity of oocyst within water may mean that the mean count of oocysts in water does not adequately represent the risk (Gale et al., 2002; Gale, 1996).

11.11 The assumption from reductions in cryptosporidiosis, particularly in the first half of the year, is that reductions have been predominantly related to the identification of water supplies at significant risk and subsequent intereventions such as the installation of additional treatment or abandonment of the source. The Drinking Water Inspectorate has put in place a process with the water industry for risk assessments to be reviewed to determine whether any aspects of current practice could be improved in light of investigations by DWI and the OCT in respect of two outbreaks in autumn 2005. When these findings are available there may be merit in carrying out a study of human disease associated with selected low and high risk supplies over a defined and relevant period of time.

11.12 **An analytical epidemiological study using geographic data on water supply and surveillance data on human *Cryptosporidium* cases should be conducted to compare the risks associated with supplies passing the risk assessment with those failing and whether oocysts have been detected in the treated water.**

11.13 Swimming pools

11.14 There is evidence that swimming pools within the UK are contributing to an increase in cryptosporidiosis within local communities in the autumn period.

Appropriate ways of examining these outbreaks need to be established so that better evidence is made available. In particular, is there a link between infections in returning tourists and subsequent swimming pool outbreaks in the UK.

11.15 There is a strong suspicion that swimming pool outbreaks in other EU states are contributing to regular late summer/autumn outbreaks in the UK.

11.16 Swimming pools appear to play an important part in the epidemiology of cryptosporidiosis in the second half of the year. While failures in filtration are likely to be important it is difficult to pinpoint the management practices and infrastructural limitations that are responsible in these outbreaks. This makes it difficult to identify interventions that may be useful in preventing further cases in an outbreak and in reducing the risks of outbreaks in subsequent years.

11.17 Outbreaks related to drinking water have declined in recent years but there are still outbreaks linked to swimming pools and farm visits.

11.18 **A protocol for the examination of swimming pool and farm related outbreaks needs to be produced so that risk related factors can be better identified.**

11.19 Travel

11.20 Travel related cryptosporidiosis have been recognised for years. The occurrence of two large outbreaks in the UK in different years associated with hotel pools in Majorca suggests that outbreaks linked to overseas resorts may be contributing significantly to the national burden of cases.

11.21 **A study into the risk factors for individuals travelling to other countries is needed to provide appropriate advice to travellers as well as to guide the government on where efforts to reduce the risk to travellers should be targeted.**

11.22 Improving laboratory detection

11.23 Evidence from a number of sources indicates that the staining methods currently used for screening human faecal samples for *Cryptosporidium* oocysts may be missing about a half of all the cases. In addition a number of laboratories adopt selection criteria for testing faecal samples that result in further cases being undiagnosed.

11.24 **Additional research needs to be conducted to develop practical but sensitive methods that can be used to screen faecal samples more reliably than the currently used staining methods.**

11.25 **All laboratories should be encouraged to use standard methods and testing criteria for examining faecal samples for *Cryptosporidium* oocysts.**

11.26 **The variation in local practice in the application of the national standard method for *Cryptosporidium* should be addressed as presently this is resulting in a non-systematic bias in detection and under-ascertainment of cases**

11.27 Speciation of isolates

11.28 The genotyping of isolates of *Cryptosporidium* has provided clear information on the changing distribution of the two main species within the human population. Because the epidemiology of the two main species (*C. hominis* and *C. parvum*) differs both in reservoirs, transmission, age distribution and seasonality it would be useful and informative to type all isolates. It has been useful in identifying species-specific risk factors. There is a strong rationale for typing all isolates and feeding the results back into routine surveillance and analytical studies.

11.29 ***Cryptosporidium* genotyping should be extended to all human isolates to enable the identified epidemiological studies to be conducted and to assist in the assessment of need for additional interventions for prevention and control. Funding needs to be found for this.**

11.30 Identifying the value of sub-typing

11.31 Sub-typing *Cryptosporidium* oocysts has generated much new information and has the potential to provide new insights into the transmission of disease.

11.32 **Research needs to be undertaken to improve the methods for genetic sub-typing of material extracted from faeces that could be used on all samples.**

11.33 **Although research is currently underway into the investigation of *C. hominis* subtypes using a variety of typing methods, further studies of the population genetics of *C. hominis* are required to allow greater differentiation between isolates.**

11.34 **Sub-typing methods for *C. parvum* and *C. hominis* should be subjected to systematic evaluation in an inter-laboratory trial.**

11.35 **The ways in which sub-typing might be useful in further elucidating the epidemiology of cryptosporidiosis need to be examined**.

11.36 Surveillance data

11.37 The usefulness of the routine cryptosporidiosis surveillance data in providing powerful insights into its epidemiology and prevention is limited by the lack of timeliness, completeness and dissemination of its findings. The surveillance data needs to include typing of all samples to main species level and incorporation of this data into national records.

11.38 The remaining differences in practice between laboratories are measured through occasional surveys of reporting practice.

11.39 The improvements in the timeliness and completeness of reporting to national surveillance have increased the ability to detect national increases in cases in a timely manner. The increase in the capture of the post-code of patients in a way that does not compromise patient confidentiality allows the ability to conduct geographic analytical studies that have not previously been possible. A recent Wellcome study organised as a collaboration between HPA and UEA has identified useful geographic approaches to the analysis of data on cryptosporidiosis cases, including case-control studies.

11.40 **Existing procedures for notification, surveillance and alerting of cases of cryptosporidiosis should be reviewed.**

11.41 **Data from *Cryptosporidium* genotyping should be fed into national surveillance in a timely way through interaction between the Cryptosporidium Reference Unit and the HPA Centre for Infections.**

11.42 **The timeliness and completeness of surveillance data needs to be further improved through the HPA Centre for Infections and HPA Local and Regional Services focussing attention on areas with poor rates of reporting.**

12 Bibliography

1. (1994). Cryptosporidium infections associated with swimming pools--Dane County, Wisconsin, 1993. MMWR Morb. Mortal. Wkly. Rep. *43*, 561-563.

2. (2001a). From the Centers for Disease Control and Prevention. Protracted outbreaks of cryptosporidiosis associated with swimming pool use--Ohio and Nebraska, 2000. JAMA *285*, 2967-2969.

3. (2001b). Protracted outbreaks of cryptosporidiosis associated with swimming pool use--Ohio and Nebraska, 2000. MMWR Morb. Mortal. Wkly. Rep. *50*, 406-410.

4. Abe,N., Sawano,Y., Yamada,K., Kimata,I., and Iseki,M. (2002). Cryptosporidium infection in dogs in Osaka, Japan. Vet. Parasitol. *108*, 185-193.

5. Abrahamsen,M.S., Templeton,T.J., Enomoto,S., Abrahante,J.E., Zhu,G., Lancto,C.A., Deng,M., Liu,C., Widmer,G., Tzipori,S., Buck,G.A., Xu,P., Bankier,A.T., Dear,P.H., Konfortov,B.A., Spriggs,H.F., Iyer,L., Anantharaman,V., Aravind,L., and Kapur,V. (2004). Complete genome sequence of the apicomplexan, Cryptosporidium parvum. Science *304*, 441-445.

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

7. Anderson,Y. (2001). A waterborne Disease outbreak. Water Sci. Technol. *24*, 13-15.

8. Anon (1996a). Foodborne outbreak of diarrheal illness associated with Cryptosporidium parvum--Minnesota, 1995. MMWR Morb. Mortal. Wkly. Rep. *45*, 783-784.

9. Anon (1996b). Outbreak of cryptosporidiosis at a day camp--Florida, July-August 1995. MMWR Morb. Mortal. Wkly. Rep. *45*, 442-444.

10. Anon (2000). Review of outbreaks of cryptosporidiosis in swimming pools and advice on proceedings of strategic workshop on viability tests and genetic typing. Final report to DEFRA: Drinking Water Inspectorate. Foundation for Water Research, Marlow, Bucks, UK (http://www.fwr.org/).).

11. Anon (2002). The development of a national collection for oocysts of *Cryptosporidium*. Final Report to DEFRA: Drinking Water Inspectorate. Foundation for Water Research, Marlow, Bucks, UK (http://www.fwr.org/).).

12. Anon (2005). Investigation of *Cryptosporidium* clinical isolates and analysis with epidemiological data. Final report to Drinking Water Inspectorate. Foundation for Water Research, Marlow, Bucks, UK (http://www.fwr.org/).).

13. Ashbolt,R.H., Coleman,D.J., Misrachi,A., Conti,J.M., and Kirk,M.D. (2003). An outbreak of cryptosporidiosis associated with an animal nursery at a regional fair. Commun. Dis. Intell. *27*, 244-249.

14. Badenoch,J. (1990). Cryptosporidium in water supplies. Report of the group of experts. (London: Department of the Environment, Department of Health), pp. 1-230.

15. Badenoch,J. (1995). Cryptosporidium in water supplies. Second report of the group of experts. (London: Department of the Environment, Department of Health).

16. Barwick,R.S., Levy,D.A., Craun,G.F., Beach,M.J., and Calderon,R.L. (2000). Surveillance for waterborne-disease outbreaks--United States, 1997-1998. MMWR CDC Surveill Summ. *49*, 1-21.

17. Bell,A., Guasparini,R., Meeds,D., Mathias,R.G., and Farley,J.D. (1993). A swimming pool-associated outbreak of Cryptosporidiosis in British Columbia. Can. J. Public Health *84*, 334-337.

18. Bier,J.W. (1991). Isolation of parasites on fruits and vegetables. Southeast Asian J. Trop. Med. Public Health *22 Suppl*, 144-145.

19. Black,R.E. (1986). Pathogens that cause travelers' diarrhea in Latin America and Africa. Rev. Infect. Dis. *8 Suppl 2*, S131-S135.

20. Bonadonna,L., Briancesco,R., Magini,V., Orsini,M., and Romano-Spica,V. (2004). [A preliminary investigation on the occurrence of protozoa in swimming pools in Italy]. Ann. Ig *16*, 709-719.

21. Bouchier,I. (1998). Cryptosporidium in water supplies; Third report of the group of experts. Department of the Environment, Transport and the Regions, Department of Health), pp. 1-171.

22. Bridgman,S.A., Robertson,R.M., Syed,Q., Speed,N., Andrews,N., and Hunter,P.R. (1995). Outbreak of cryptosporidiosis associated with a disinfected groundwater supply. Epidemiol. Infect. *115*, 555-566.

23. Brookes,J.D., Hipsey,M.R., Burch,M.D., Regel,R.H., Linden,L.G., Ferguson,C.M., and Antenucci,J.P. (2005). Relative value of surrogate indicators for detecting pathogens in lakes and reservoirs. Environ. Sci. Technol. *39*, 8614-8621.

24. Caccio,S. and Pozio,E. (2001). Molecular identification of food-borne and water-borne protozoa. Southeast Asian J. Trop. Med. Public Health *32 Suppl 2*, 156-158.

25. Carey,C.M., Lee,H., and Trevors,J.T. (2004). Biology, persistence and detection of Cryptosporidium parvum and Cryptosporidium hominis oocyst. Water Res. *38*, 818-862.

26. Cartwright,R.Y. (2003). Food and waterborne infections associated with package holidays. J. Appl. Microbiol. *94 Suppl*, 12S-24S.

27. Casemore,D.P., Sands,R.L., and Curry,A. (1985). Cryptosporidium species a "new" human pathogen. J. Clin. Pathol. *38*, 1321-1336.

28. Causer,L.M., Handzel,T., Welch,P., Carr,M., Culp,D., Lucht,R., Mudahar,K., Robinson,D., Neavear,E., Fenton,S., Rose,C., Craig,L., Arrowood,M., Wahlquist,S., Xiao,L., Lee,Y.M., Mirel,L., Levy,D., Beach,M.J., Poquette,G., and Dworkin,M.S. (2006). An outbreak of Cryptosporidium hominis infection at an Illinois recreational waterpark. Epidemiol. Infect. *134*, 147-156.

29. Chalmers,R.M., Elwin,K., Reilly,W.J., Irvine,H., Thomas,A.L., and Hunter,P.R. (2002a). Cryptosporidium in farmed animals: the detection of a novel isolate in sheep. Int. J. Parasitol. *32*, 21-26.

30. Chalmers,R.M., Elwin,K., Thomas,A.L., and Joynson,D.H. (2002b). Infection with unusual types of Cryptosporidium is not restricted to immunocompromised patients. J. Infect. Dis. *185*, 270-271.

31. Chalmers,R.M., Ferguson,C., Caccio,S., Gasser,R.B., Abs El-Osta,Y.G., Heijnen,L., Xiao,L., Elwin,K., Hadfield,S., Sinclair,M., and Stevens,M. (2005). Direct comparison of selected methods for genetic categorisation of Cryptosporidium parvum and Cryptosporidium hominis species. Int. J. Parasitol. *35*, 397-410.

32. Chalmers,R.M., Hughes,S., Thomas,A.L., Woodhouse,S., Thomas,P.D., and Hunter,P. (2002c). Laboratory ascertainment of Cryptosporidium and local authority policies for investigating sporadic cases of cryptosporidiosis in two regions of the United Kingdom. Commun. Dis. Public Health *5*, 114-118.

33. Chalmers,R.M., Sturdee,A.P., Bull,S.A., Miller,A., and Wright,S.E. (1997a). The prevalence of Cryptosporidium parvum and C. muris in Mus domesticus, Apodemus sylvaticus and Clethrionomys glareolus in an agricultural system. Parasitol. Res. *83*, 478-482.

34. Chalmers,R.M., Sturdee,A.P., Mellors,P., Nicholson,V., Lawlor,F., Kenny,F., and Timpson,P. (1997b). Cryptosporidium parvum in environmental samples in the Sligo area, Republic of Ireland: a preliminary report. Lett. Appl. Microbiol. *25*, 380-384.

35. Chappell,C.L., Okhuysen,P.C., Sterling,C.R., and DuPont,H.L. (1996). Cryptosporidium parvum: intensity of infection and oocyst excretion patterns in healthy volunteers. J. Infect. Dis. *173*, 232-236.

36. Chappell,C.L., Okhuysen,P.C., Sterling,C.R., Wang,C., Jakubowski,W., and DuPont,H.L. (1999). Infectivity of Cryptosporidium parvum in healthy adults

with pre-existing anti-C. parvum serum immunoglobulin G. Am. J. Trop. Med. Hyg. *60*, 157-164.

37. Cirak,V.Y. and Bauer,C. (2004). Comparison of conventional coproscopical methods and commercial coproantigen ELISA kits for the detection of Giardia and Cryptosporidium infections in dogs and cats. Berl Munch. Tierarztl. Wochenschr. *117*, 410-413.

38. Collins,M.V., Flick,G.J., Smith,S.A., Fayer,R., Croonenberghs,R., O'Keefe,S., and Lindsay,D.S. (2005a). The effect of high-pressure processing on infectivity of Cryptosporidium parvum oocysts recovered from experimentally exposed Eastern oysters (Crassostrea virginica). J. Eukaryot. Microbiol. *52*, 500-504.

39. Collins,M.V., Flick,G.J., Smith,S.A., Fayer,R., Rubendall,E., and Lindsay,D.S. (2005b). The effects of E-beam irradiation and microwave energy on Eastern Oysters (Crassostrea virginica) experimentally infected with Cryptosporidium parvum. J. Eukaryot. Microbiol. *52*, 484-488.

40. Cook,N. (2003). The use of NASBA for the detection of microbial pathogens in food and environmental samples. J. Microbiol. Methods *53*, 165-174.

41. Cooper,D.L., Verlander,N.Q., Smith,G.E., Charlett,A., Gerard,E., Willocks,L., and O'Brien,S. (2006). Can syndromic surveillance data detect local outbreaks of communicable disease? A model using a historical cryptosporidiosis outbreak. Epidemiol. Infect. *134*, 13-20.

42. Craven,D.E., Steger,K.A., and Hirschhorn,L.R. (1996). Nosocomial colonization and infection in persons infected with human immunodeficiency virus. Infect. Control Hosp. Epidemiol. *17*, 304-318.

43. Cruickshank,R., Ashdown,L., and Croese,J. (1988). Human cryptosporidiosis in North Queensland. Aust. N. Z. J. Med. *18*, 582-586.

44. Current,W.L. and Reese,N.C. (1986). A comparison of endogenous development of three isolates of Cryptosporidium in suckling mice. J. Protozool. *33*, 98-108.

45. Curriero,F.C., Patz,J.A., Rose,J.B., and Lele,S. (2001). The association between extreme precipitation and waterborne disease outbreaks in the United States, 1948-1994. Am. J. Public Health *91*, 1194-1199.

46. D'Antonio,R.G., Winn,R.E., Taylor,J.P., Gustafson,T.L., Current,W.L., Rhodes,M.M., Gary,G.W., Jr., and Zajac,R.A. (1985). A waterborne outbreak of cryptosporidiosis in normal hosts. Ann. Intern. Med. *103*, 886-888.

47. Dai,X. and Boll,J. (2006). Settling velocity of Cryptosporidium parvum and Giardia lamblia. Water Res. *40*, 1321-1325.

48. Dawson,A., Griffin,R., Fleetwood,A., and Barrett,N.J. (1995). Farm visits and zoonoses. Commun. Dis. Rep. CDR Rev. *5*, R81-R86.

49. Dawson,D. (2005). Foodborne protozoan parasites. Int. J. Food Microbiol. *103*, 207-227.

50. Dawson,D.J., Samuel,C.M., Scrannage,V., and Atherton,C.J. (2004). Survival of Cryptosporidium species in environments relevant to foods and beverages. J. Appl. Microbiol. *96*, 1222-1229.

51. Deng,M.Q. and Cliver,D.O. (2000). Comparative detection of Cryptosporidium parvum oocysts from apple juice. Int. J. Food Microbiol. *54*, 155-162.

52. Deng,M.Q., Lam,K.M., and Cliver,D.O. (2000). Immunomagnetic separation of Cryptosporidium parvum oocysts using MACS MicroBeads and high gradient separation columns. J. Microbiol. Methods *40*, 11-17.

53. DiGiorgio,C.L., Gonzalez,D.A., and Huitt,C.C. (2002). Cryptosporidium and giardia recoveries in natural waters by using environmental protection agency method 1623. Appl. Environ. Microbiol. *68*, 5952-5955.

54. Dillingham,R.A., Lima,A.A., and Guerrant,R.L. (2002). Cryptosporidiosis: epidemiology and impact. Microbes. Infect. *4*, 1059-1066.

55. Djuretic,T., Wall,P.G., and Nichols,G. (1997). General outbreaks of infectious intestinal disease associated with milk and dairy products in England and Wales: 1992 to 1996. Commun. Dis. Rep. CDR Rev. *7*, R41-R45.

56. Duffy,G. and Moriarty,E.M. (2003). Cryptosporidium and its potential as a food-borne pathogen. Anim Health Res. Rev. *4*, 95-107.

57. Duke,L.A., Breathnach,A.S., Jenkins,D.R., Harkis,B.A., and Codd,A.W. (1996). A mixed outbreak of cryptosporidium and campylobacter infection associated with a private water supply. Epidemiol. Infect. *116*, 303-308.

58. DuPont,H.L., Chappell,C.L., Sterling,C.R., Okhuysen,P.C., Rose,J.B., and Jakubowski,W. (1995). The infectivity of Cryptosporidium parvum in healthy volunteers. N. Engl. J. Med. *332*, 855-859.

59. Ederli,B.B., Rodrigues,M.F., and Carvalho,C.B. (2005). [Oocysts of the genus Cryptosporidium in domiciliated dogs from the city of Campos dos Goytacazes, the State of Rio de Janeiro]. Rev. Bras. Parasitol. Vet. *14*, 129-131.

60. el Sibaei,M.M., Rifaat,M.M., Hameed,D.M., and el Din,H.M. (2003). Nosocomial sources of cryptosporidial infection in newly admitted patients in Ain Shams University Pediatric Hospital. J. Egypt. Soc. Parasitol. *33*, 177-188.

61. Elwin,K., Chalmers,R.M., Roberts,R., Guy,E.C., and Casemore,D.P. (2001). Modification of a rapid method for the identification of gene-specific polymorphisms in Cryptosporidium parvum and its application to clinical and epidemiological investigations. Appl. Environ. Microbiol. *67*, 5581-5584.

62. European Union (2006). Directive 2006/7/EC of the European Parliament and of the Council of 15 February 2006 concerning the management of bathing water quality and repealing Directive 76/160/EEC. Official Journal of the European Union *L64*, 37-51.

63. Evans,M.R. and Gardner,D. (1996). Cryptosporidiosis outbreak associated with an educational farm holiday. Commun. Dis. Rep. CDR Rev. *6*, R50-R51.

64. Fontanarrosa,M.F., Vezzani,D., Basabe,J., and Eiras,D.F. (2006). An epidemiological study of gastrointestinal parasites of dogs from Southern Greater Buenos Aires (Argentina): age, gender, breed, mixed infections, and seasonal and spatial patterns. Vet. Parasitol. *136*, 283-295.

65. Franco,R.M. and Cantusio,N.R. (2002). Occurrence of cryptosporidial oocysts and giardia cysts in bottled mineral water commercialized in the city of Campinas, State of Sao Paulo, Brazil. Mem. Inst. Oswaldo Cruz *97*, 205-207.

66. Frost,F.J., Fea,E., Gilli,G., Biorci,F., Muller,T.M., Craun,G.F., and Calderon,R.L. (2000). Serological evidence of Cryptosporidium infections in southern Europe. Eur. J. Epidemiol. *16*, 385-390.

67. Frost,F.J., Roberts,M., Kunde,T.R., Craun,G., Tollestrup,K., Harter,L., and Muller,T. (2005). How clean must our drinking water be: the importance of protective immunity. J. Infect. Dis. *191*, 809-814.

68. Gale,P. (1996). Developments in microbiological risk assessment models for drinking water--a short review. J. Appl. Bacteriol. *81*, 403-410.

69. Gale,P., Pitchers,R., and Gray,P. (2002). The effect of drinking water treatment on the spatial heterogeneity of micro-organisms: implications for assessment of treatment efficiency and health risk. Water Res. *36*, 1640-1648.

70. Garcia,L., Henderson,J., Fabri,M., and Oke,M. (2006). Potential sources of microbial contamination in unpasteurized apple cider. J. Food Prot. *69*, 137-144.

71. Gardner,C. (1994). An outbreak of hospital-acquired cryptosporidiosis. Br. J. Nurs. *3*, 152, 154-152, 158.

72. Gelletlie,R., Stuart,J., Soltanpoor,N., Armstrong,R., and Nichols,G. (1997). Cryptosporidiosis associated with school milk. Lancet *350*, 1005-1006.

73. Glaberman,S., Moore,J.E., Lowery,C.J., Chalmers,R.M., Sulaiman,I., Elwin,K., Rooney,P.J., Millar,B.C., Dooley,J.S., Lal,A.A., and Xiao,L. (2002). Three drinking-water-associated cryptosporidiosis outbreaks, Northern Ireland. Emerg. Infect. Dis. *8*, 631-633.

74. Goh,S., Reacher,M., Casemore,D.P., Verlander,N.Q., Chalmers,R., Knowles,M., Williams,J., Osborn,K., and Richards,S. (2004). Sporadic cryptosporidiosis, North Cumbria, England, 1996-2000. Emerg. Infect. Dis. *10*, 1007-1015.

75. Goh,S., Reacher,M., Casemore,D.P., Verlander,N.Q., Charlett,A., Chalmers,R.M., Knowles,M., Pennington,A., Williams,J., Osborn,K., and Richards,S. (2005). Sporadic cryptosporidiosis decline after membrane filtration of public water supplies, England, 1996-2002. Emerg. Infect. Dis. *11*, 251-259.

76. Graczyk,T.K., Cranfield,M.R., Fayer,R., and Bixler,H. (1999a). House flies (Musca domestica) as transport hosts of Cryptosporidium parvum. Am. J. Trop. Med. Hyg. *61*, 500-504.

77. Graczyk,T.K., Fayer,R., Cranfield,M.R., Mhangami-Ruwende,B., Knight,R., Trout,J.M., and Bixler,H. (1999b). Filth flies are transport hosts of Cryptosporidium parvum. Emerg. Infect. Dis. *5*, 726-727.

78. Graczyk,T.K., Fayer,R., Knight,R., Mhangami-Ruwende,B., Trout,J.M., da Silva,A.J., and Pieniazek,N.J. (2000). Mechanical transport and transmission of Cryptosporidium parvum oocysts by wild filth flies. Am. J. Trop. Med. Hyg. *63*, 178-183.

79. Graczyk,T.K. and Schwab,K.J. (2000). Foodborne infections vectored by molluscan shellfish. Curr. Gastroenterol. Rep. *2*, 305-309.

80. Greinert,J., Furtado,D., Smith,J., Monte,B.C., and Simoes,C. (2004). Detection of Cryptosporidium oocysts and Giardia cysts in swimming pool filter backwash water concentrates by flocculation and immunomagnetic separation. Int. J. Environ. Health Res. *14*, 395-404.

81. Gutteridge,W. and Haworth,E.A. (1994). An outbreak of gastrointestinal illness associated with contamination of the mains supply by river water. Commun. Dis. Rep. CDR. Wkly. *CDR Review. 4*, R50-1.

82. Hackett,T. and Lappin,M.R. (2003). Prevalence of enteric pathogens in dogs of north-central Colorado. J. Am. Anim Hosp. Assoc. *39*, 52-56.

83. Hajdusek,O., Ditrich,O., and Slapeta,J. (2004). Molecular identification of Cryptosporidium spp. in animal and human hosts from the Czech Republic. Vet. Parasitol. *122*, 183-192.

84. Hall, T., Irving, T., Nichols, G., Nichols, T., Hunter, P. R., Reacher, M., Lane, C., and Smerdon, W. Risk reduction and reassessment for sites that require continuous monitoring for cryptosporidium. 2002. Foundation for Water Research.

85. Hanes,D.E., Worobo,R.W., Orlandi,P.A., Burr,D.H., Miliotis,M.D., Robl,M.G., Bier,J.W., Arrowood,M.J., Churey,J.J., and Jackson,G.J. (2002). Inactivation of Cryptosporidium parvum oocysts in fresh apple cider by UV irradiation. Appl. Environ. Microbiol. *68*, 4168-4172.

86. Hannah,J. and Riordan,T. (1988). Case to case spread of cryptosporidiosis; evidence from a day nursery outbreak. Public Health *102*, 539-544.

87. Hansen,J.S. and Ongerth,J.E. (1991). Effects of time and watershed characteristics on the concentration of Cryptosporidium oocysts in river water. Appl. Environ. Microbiol. *57*, 2790-2795.

88. Harrison,S.L., Nelder,R., Hayek,L., Mackenzie,I.F., Casemore,D.P., and Dance,D. (2002). Managing a large outbreak of cryptosporidiosis: how to investigate and when to decide to lift a 'boil water' notice. Commun. Dis. Public Health *5*, 230-239.

89. Hayes,E.B., Matte,T.D., O'Brien,T.R., McKinley,T.W., Logsdon,G.S., Rose,J.B., Ungar,B.L., Word,D.M., Pinsky,P.F., Cummings,M.L., and . (1989). Large community outbreak of cryptosporidiosis due to contamination of a filtered public water supply. N. Engl. J. Med. *320*, 1372-1376.

90. Hellard,M., Hocking,J., Willis,J., Dore,G., and Fairley,C. (2003). Risk factors leading to Cryptosporidium infection in men who have sex with men. Sex Transm. Infect. *79*, 412-414.

91. Henriksen,S.A. and Pohlenz,J.F. (1981). Staining of cryptosporidia by a modified Ziehl-Neelsen technique. Acta Vet. Scand. *22*, 594-596.

92. Hiepe,T. and Buchwalder,R. (1991). [Livestock manure as a vector for parasites--a report of experiences]. Dtsch. Tierarztl. Wochenschr. *98*, 268-272.

93. Hommer,V., Eichholz,J., and Petry,F. (2003). Effect of antiretroviral protease inhibitors alone, and in combination with paromomycin, on the excystation, invasion and in vitro development of Cryptosporidium parvum. J. Antimicrob. Chemother. *52*, 359-364.

94. Hrudy,S.E. and Hrudy,E.J. (2004). Safe Drinking water: Lessons from recent outbreaks in affluent nations. (London: IWA), pp. 1-486.

95. Hsu,B.M. and Yeh,H.H. (2003). Removal of Giardia and Cryptosporidium in drinking water treatment: a pilot-scale study. Water Res. *37*, 1111-1117.

96. Huang,J., Mullapudi,N., Sicheritz-Ponten,T., and Kissinger,J.C. (2004). A first glimpse into the pattern and scale of gene transfer in Apicomplexa. Int. J. Parasitol. *34*, 265-274.

97. Huber,F., Bomfim,T.C., and Gomes,R.S. (2005). Comparison between natural infection by Cryptosporidium sp., Giardia sp. in dogs in two living situations in the West Zone of the municipality of Rio de Janeiro. Vet. Parasitol. *130*, 69-72.

98. Hunt,D.A., Sebugwawo,S., Edmondson,S.G., and Casemore,D.P. (1994). Cryptosporidiosis associated with a swimming pool complex. Commun. Dis. Rep. CDR Rev. *4*, R20-R22.

99. Hunter,P., 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. Commun. Dis. Public Health *4*, 136-138.

100. 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. Emerg. Infect. Dis. *9*, 109-112.

101. Hunter,P.R., Hughes,S., Woodhouse,S., Syed,Q., Verlander,N.Q., Chalmers,R.M., Morgan,K., Nichols,G., Beeching,N., and Osborn,K. (2004). Sporadic cryptosporidiosis case-control study with genotyping. Emerg. Infect. Dis. *10*, 1241-1249.

102. Hunter,P.R. and Nichols,G. (2002). Epidemiology and clinical features of Cryptosporidium infection in immunocompromised patients. Clin. Microbiol. Rev. *15*, 145-154.

103. Hunter,P.R. and Syed,Q. (2002). A community survey of self-reported gastroenteritis undertaken during an outbreak of cryptosporidiosis strongly associated with drinking water after much press interest. Epidemiol. Infect. *128*, 433-438.

104. Insulander,M., Lebbad,M., Stenstrom,T.A., and Svenungsson,B. (2005). An outbreak of cryptosporidiosis associated with exposure to swimming pool water. Scand. J. Infect. Dis. *37*, 354-360.

105. Ives,N.J., Gazzard,B.G., and Easterbrook,P.J. (2001). The changing pattern of AIDS-defining illnesses with the introduction of highly active antiretroviral therapy (HAART)in a London clinic. J. Infect. *42*, 134-139.

106. Jephcott,A.E., Begg,N.T., and Baker,I.A. (1986). Outbreak of giardiasis associated with mains water in the United Kingdom. Lancet *1*, 730-732.

107. Joce,R.E., Bruce,J., Kiely,D., Noah,N.D., Dempster,W.B., Stalker,R., Gumsley,P., Chapman,P.A., Norman,P., Watkins,J., and . (1991). An outbreak of cryptosporidiosis associated with a swimming pool. Epidemiol. Infect. *107*, 497-508.

108. Jokipii,L., Pohjola,S., and Jokipii,A.M. (1985). Cryptosporidiosis associated with traveling and giardiasis. Gastroenterology *89*, 838-842.

109. Jones,M., Boccia,D., Kealy,M., Salkin,B., Ferrero,A., Nichols,G., and Stuart,J. (2006). Cryptosporidium outbreak linked to interactive water feature, UK: importance of guidelines. Euro. Surveill *11*.

110. Jougla,E., Pequignot,F., Carbon,C., Pavillon,G., Mireille,E.B., Bourdais,J.P., Bourdais,O., and Hatton,F. (1996). AIDS-related conditions: study of a representative sample of 1203 patients deceased in 1992 in France. Int. J. Epidemiol. *25*, 190-197.

111. Khalakdina,A., Vugia,D.J., Nadle,J., Rothrock,G.A., and Colford,J.M., Jr. (2003). Is drinking water a risk factor for endemic cryptosporidiosis? A case-control study in the immunocompetent general population of the San Francisco Bay Area. BMC. Public Health *3*, 11.

112. Kim,L.S., Stansell,J., Cello,J.P., and Koch,J. (1998). Discrepancy between sex- and water-associated risk behaviors for cryptosporidiosis among HIV-infected patients in San Francisco. J. Acquir. Immune. Defic. Syndr. Hum. Retrovirol. *19*, 44-49.

113. Kniel,K.E. and Jenkins,M.C. (2005). Detection of Cryptosporidium parvum oocysts on fresh vegetables and 1 herbs using antibodies specific for a Cryptosporidium parvum viral antigen. J. Food Prot. *68*, 1093-1096.

114. Kniel,K.E., Sumner,S.S., Lindsay,D.S., Hackney,C.R., Pierson,M.D., Zajac,A.M., Golden,D.A., and Fayer,R. (2003). Effect of organic acids and hydrogen peroxide on Cryptosporidium parvum viability in fruit juices. J. Food Prot. *66*, 1650-1657.

115. Kramer,M.H., Sorhage,F.E., Goldstein,S.T., Dalley,E., Wahlquist,S.P., and Herwaldt,B.L. (1998). First reported outbreak in the United States of cryptosporidiosis associated with a recreational lake. Clin. Infect. Dis. *26*, 27-33.

116. Laberge,I., Ibrahim,A., Barta,J.R., and Griffiths,M.W. (1996). Detection of Cryptosporidium parvum in raw milk by PCR and oligonucleotide probe hybridization. Appl. Environ. Microbiol. *62*, 3259-3264.

117. Lake,I.R., Bentham,G., Kovats,R.S., and Nichols,G.L. (2005). Effects of weather and river flow on cryptosporidiosis. J. Water Health *3*, 469-474.

118. Lallo,M.A. and Bondan,E.F. (2006). [Prevalence of Cryptosporidium sp. in institutionalized dogs in the city of Sao Paulo, Brazil]. Rev. Saude Publica *40*, 120-125.

119. Leach,C.T., Koo,F.C., Kuhls,T.L., Hilsenbeck,S.G., and Jenson,H.B. (2000). Prevalence of Cryptosporidium parvum infection in children along the Texas-Mexico border and associated risk factors. Am. J. Trop. Med. Hyg. *62*, 656-661.

120. Lee,S.H., Levy,D.A., Craun,G.F., Beach,M.J., and Calderon,R.L. (2002). Surveillance for waterborne-disease outbreaks--United States, 1999-2000. MMWR Surveill Summ. *51*, 1-47.

121. Lemmon,J.M., McAnulty,J.M., and Bawden-Smith,J. (1996). Outbreak of cryptosporidiosis linked to an indoor swimming pool. Med. J. Aust. *165*, 613-616.

122. Leoni,F., Amar,C., Nichols,G., Pedraza-Diaz,S., and McLauchlin,J. (2006). Genetic analysis of Cryptosporidium from 2414 humans with diarrhoea in England between 1985 and 2000. J. Med. Microbiol. *55*, 703-707.

123. Leoni,F., Gallimore,C.I., Green,J., and McLauchlin,J. (2003). Molecular epidemiological analysis of Cryptosporidium isolates from humans and animals by using a heteroduplex mobility assay and nucleic acid sequencing based on a small double-stranded RNA element. J. Clin. Microbiol. *41*, 981-992.

124. Li,X., Guyot,K., Dei-Cas,E., Mallard,J.P., Ballet,J.J., and Brasseur,P. (2006). Cryptosporidium oocysts in mussels (Mytilus edulis) from Normandy (France). Int. J. Food Microbiol. *108*, 321-325.

125. Lim,L.S., Varkey,P., Giesen,P., and Edmonson,L. (2004). Cryptosporidiosis outbreak in a recreational swimming pool in Minnesota. J. Environ. Health *67*, 16-20, 28, 27.

126. Liu,C., Vigdorovich,V., Kapur,V., and Abrahamsen,M.S. (1999). A random survey of the Cryptosporidium parvum genome. Infect. Immun. *67*, 3960-3969.

127. Lloyd,A. and Drury,D. (2002). Continuous monitoring for Cryptosporidium--a novel approach to public health protection. Water Sci. Technol. *46*, 297-301.

128. Lopez-Velez,R., Tarazona,R., Garcia,C.A., Gomez-Mampaso,E., Guerrero,A., Moreira,V., and Villanueva,R. (1995). Intestinal and extraintestinal cryptosporidiosis in AIDS patients. Eur. J. Clin. Microbiol. Infect. Dis. *14*, 677-681.

129. Louie,K., Gustafson,L., Fyfe,M., Gill,I., MacDougall,L., Tom,L., Wong,Q., and Isaac-Renton,J. (2004). An outbreak of Cryptosporidium parvum in a Surrey pool with detection in pool water sampling. Can. Commun. Dis. Rep. *30*, 61-66.

130. Mac Kenzie,W.R., Hoxie,N.J., Proctor,M.E., Gradus,M.S., Blair,K.A., Peterson,D.E., Kazmierczak,J.J., Addiss,D.G., Fox,K.R., Rose,J.B., and . (1994). A massive outbreak in Milwaukee of cryptosporidium infection transmitted through the public water supply. N. Engl. J. Med. *331*, 161-167.

131. Mackenzie,W.R., Kazmierczak,J.J., and Davis,J.P. (1995). An outbreak of cryptosporidiosis associated with a resort swimming pool. Epidemiol. Infect. *115*, 545-553.

132. MacRae,M., Hamilton,C., Strachan,N.J., Wright,S., and Ogden,I.D. (2005). The detection of Cryptosporidium parvum and Escherichia coli O157 in UK bivalve shellfish. J. Microbiol. Methods *60*, 395-401.

133. Mahdi,N.K. and Ali,N.H. (2002). Cryptosporidiosis among animal handlers and their livestock in Basrah, Iraq. East Afr. Med. J. *79*, 550-553.

134. Mallon,M., Macleod,A., Wastling,J., Smith,H., Reilly,B., and Tait,A. (2003a). Population structures and the role of genetic exchange in the zoonotic pathogen Cryptosporidium parvum. J. Mol. Evol. *56*, 407-417.

135. Mallon,M.E., Macleod,A., Wastling,J.M., Smith,H., and Tait,A. (2003b). Multilocus genotyping of Cryptosporidium parvum Type 2: population genetics and sub-structuring. Infect. Genet. Evol. *3*, 207-218.

136. Manfredi,R. (2000). [Effects of the introduction of highly active antiretroviral therapy (HAART) on the temporal trend of diagnosis of AIDS and AIDS-defining disorders]. Infez. Med. *8*, 134-138.

137. Martino,P., Gentile,G., Caprioli,A., Baldassarri,L., Donelli,G., Arcese,W., Fenu,S., Micozzi,A., Venditti,M., and Mandelli,F. (1988). Hospital-acquired cryptosporidiosis in a bone marrow transplantation unit. J. Infect. Dis. *158*, 647-648.

138. Mathieu,E., Levy,D.A., Veverka,F., Parrish,M.K., Sarisky,J., Shapiro,N., Johnston,S., Handzel,T., Hightower,A., Xiao,L., Lee,Y.M., York,S., Arrowood,M., Lee,R., and Jones,J.L. (2004). Epidemiologic and environmental investigation of a recreational water outbreak caused by two genotypes of Cryptosporidium parvum in Ohio in 2000. Am. J. Trop. Med. Hyg. *71*, 582-589.

139. McAnulty,J.M., Fleming,D.W., and Gonzalez,A.H. (1994). A community-wide outbreak of cryptosporidiosis associated with swimming at a wave pool. JAMA *272*, 1597-1600.

140. McGlade,T.R., Robertson,I.D., Elliot,A.D., Read,C., and Thompson,R.C. (2003). Gastrointestinal parasites of domestic cats in Perth, Western Australia. Vet. Parasitol. *117*, 251-262.

141. 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. J. Clin. Microbiol. *38*, 3984-3990.

142. McLauchlin,J., Amar,C.F., Pedraza-Diaz,S., Mieli-Vergani,G., Hadzic,N., and Davies,E.G. (2003). Polymerase chain reaction-based diagnosis of infection with Cryptosporidium in children with primary immunodeficiencies. Pediatr. Infect. Dis. J. *22*, 329-335.

143. McLauchlin,J., Casemore,D.P., Moran,S., and Patel,S. (1998). The epidemiology of cryptosporidiosis: application of experimental sub-typing and antibody detection systems to the investigation of water-borne outbreaks. Folia Parasitol. (Praha) *45*, 83-92.

144. McLauchlin,J., Pedraza-Diaz,S., Amar-Hoetzeneder,C., and Nichols,G.L. (1999). Genetic characterization of Cryptosporidium strains from 218 patients with diarrhea diagnosed as having sporadic cryptosporidiosis. J. Clin. Microbiol. *37*, 3153-3158.

145. Medema,G.J., Schets,F.M., Teunis,P.F., and Havelaar,A.H. (1998). Sedimentation of free and attached Cryptosporidium oocysts and Giardia cysts in water. Appl. Environ. Microbiol. *64*, 4460-4466.

146. Meisel,J.L., Perera,D.R., Meligro,C., and Rubin,C.E. (1976). Overwhelming watery diarrhea associated with a cryptosporidium in an immunosuppressed patient. Gastroenterology *70*, 1156-1160.

147. Messner,M.J., Chappell,C.L., and Okhuysen,P.C. (2001). Risk assessment for Cryptosporidium: a hierarchical Bayesian analysis of human dose response data. Water Res. *35*, 3934-3940.

148. Millard,P.S., Gensheimer,K.F., Addiss,D.G., Sosin,D.M., Beckett,G.A., Houck-Jankoski,A., and Hudson,A. (1994). An outbreak of cryptosporidiosis from fresh-pressed apple cider. JAMA *272*, 1592-1596.

149. Millson,M., Bokhout,M., Carlson,J., Aldis,R., Borczyk,A., and Loir,H. (2006). An outbreak of *Campylobacter jejuni* gastroenteritis linked to meltwater contamination of municipal well. Can. J. Public Health *82*, 27-31.

150. Moore,A.C., Herwaldt,B.L., Craun,G.F., Calderon,R.L., Highsmith,A.K., and Juranek,D.D. (1993). Surveillance for waterborne disease outbreaks--United States, 1991-1992. MMWR CDC Surveill Summ. *42*, 1-22.

151. Morgan-Ryan,U.M., Fall,A., Ward,L.A., Hijjawi,N., Sulaiman,I., Fayer,R., Thompson,R.C., Olson,M., Lal,A., and Xiao,L. (2002). Cryptosporidium hominis n. sp. (Apicomplexa: Cryptosporidiidae) from Homo sapiens. J. Eukaryot. Microbiol. *49*, 433-440.

152. Moriarty,E.M., Duffy,G., McEvoy,J.M., Caccio,S., Sheridan,J.J., McDowell,D., and Blair,I.S. (2005). The effect of thermal treatments on the viability and infectivity of Cryptosporidium parvum on beef surfaces. J. Appl. Microbiol. *98*, 618-623.

153. Moss,D.M., Bennett,S.N., Arrowood,M.J., Wahlquist,S.P., and Lammie,P.J. (1998). Enzyme-linked immunoelectrotransfer blot analysis of a cryptosporidiosis outbreak on a United States Coast Guard cutter. Am. J. Trop. Med. Hyg. *58*, 110-118.

154. Mtambo,M.M., Nash,A.S., Blewett,D.A., Smith,H.V., and Wright,S. (1991). Cryptosporidium infection in cats: prevalence of infection in domestic and feral cats in the Glasgow area. Vet. Rec. *129*, 502-504.

155. Nannini,E.C. and Okhuysen,P.C. (2002). HIV1 and the gut in the era of highly active antiretroviral therapy. Curr. Gastroenterol. Rep. *4*, 392-398.

156. Naumova,E.N., Egorov,A.I., Morris,R.D., and Griffiths,J.K. (2003). The elderly and waterborne Cryptosporidium infection: gastroenteritis hospitalizations before and during the 1993 Milwaukee outbreak. Emerg. Infect. Dis. *9*, 418-425.

157. Nichols,G. (1992). The Biology, Epidemiology and Typing of *Cryptosporidium* spp. PhD Thesis. Surrey University), pp. 1-398.

158. Nichols,G. (2003). Using existing surveillance based data. In Drinking Water and Infectious Disease – Establishing the links., P.R.Hunter, M.Wait, and E.Ronchi, eds., pp. 131-141.

159. Nichols,G. (2006). Infection risks from water in natural and man-made environments. Euro. Surveill *11*.

160. Nichols,G. and McLauchlin,J. (2003). Microbiology and the investigation of waterborne outbreaks: The use of *Cryptosporidium* typing in the investigation of waterborne disease. In Drinking Water and Infectious Disease – Establishing the links., P.R.Hunter, M.Wait, and E.Ronchi, eds., pp. 87-95.

161. Nichols,G.L. (2000). Food-borne protozoa. Br. Med. Bull. *56*, 209-235.

162. Nichols,G.L. (2005). Fly transmission of Campylobacter. Emerg. Infect. Dis. *11*, 361-364.

163. Nichols,G.L., McLauchlin,J., and Samuel,D. (1991). A technique for typing Cryptosporidium isolates. J. Protozool. *38*, 237S-240S.

164. Nichols,G.L. and Thom,B.T. (1984). Screening for Cryptosporidium in stools. Lancet *1*, 734-735.

165. Nime,F.A., Burek,J.D., Page,D.L., Holscher,M.A., and Yardley,J.H. (1976). Acute enterocolitis in a human being infected with the protozoan Cryptosporidium. Gastroenterology *70*, 592-598.

166. Okhuysen,P.C., Chappell,C.L., Crabb,J.H., Sterling,C.R., and DuPont,H.L. (1999). Virulence of three distinct Cryptosporidium parvum isolates for healthy adults. J. Infect. Dis. *180*, 1275-1281.

167. Okhuysen,P.C., Chappell,C.L., Sterling,C.R., Jakubowski,W., and DuPont,H.L. (1998). Susceptibility and serologic response of healthy adults to reinfection with Cryptosporidium parvum. Infect. Immun. *66*, 441-443.

168. Patel,S., Pedraza-Diaz,S., and McLauchlin,J. (1999). The identification of Cryptosporidium species and Cryptosporidium parvum directly from whole faeces by analysis of a multiplex PCR of the 18S rRNA gene and by PCR/RFLP of the Cryptosporidium outer wall protein (COWP) gene. Int. J. Parasitol. *29*, 1241-1247.

169. Patel,S., Pedraza-Diaz,S., McLauchlin,J., and Casemore,D.P. (1998). Molecular characterisation of Cryptosporidium parvum from two large suspected waterborne outbreaks. Outbreak Control Team South and West Devon 1995, Incident Management Team and Further Epidemiological and Microbiological Studies Subgroup North Thames 1997. Commun. Dis. Public Health *1*, 231-233.

170. Pedersen,C., Danner,S., Lazzarin,A., Glauser,M.P., Weber,R., Katlama,C., Barton,S.E., and Lundgren,J.D. (1996). Epidemiology of cryptosporidiosis among European AIDS patients. Genitourin. Med. *72*, 128-131.

171. Pedraza-Diaz,S., Amar,C., Iversen,A.M., Stanley,P.J., and McLauchlin,J. (2001a). Unusual cryptosporidium species recovered from human faeces: first description of Cryptosporidium felis and Cryptosporidium 'dog type' from patients in England. J. Med. Microbiol. *50*, 293-296.

172. Pedraza-Diaz,S., Amar,C.F., McLauchlin,J., Nichols,G.L., Cotton,K.M., Godwin,P., Iversen,A.M., Milne,L., Mulla,J.R., Nye,K., Panigrahl,H.,

Venn,S.R., Wiggins,R., Williams,M., and Youngs,E.R. (2001b). Cryptosporidium meleagridis from humans: molecular analysis and description of affected patients. J. Infect. *42*, 243-250.

173. Peng,M.M., Xiao,L., Freeman,A.R., Arrowood,M.J., Escalante,A.A., Weltman,A.C., Ong,C.S., Mac Kenzie,W.R., Lal,A.A., and Beard,C.B. (1997). Genetic polymorphism among Cryptosporidium parvum isolates: evidence of two distinct human transmission cycles. Emerg. Infect. Dis. *3*, 567-573.

174. Piper,M.B., Bankier,A.T., and Dear,P.H. (1998). A HAPPY map of Cryptosporidium parvum. Genome Res. *8*, 1299-1307.

175. Pohjola,S., Oksanen,H., Jokipii,L., and Jokipii,A.M. (1986). Outbreak of cryptosporidiosis among veterinary students. Scand. J. Infect. Dis. *18*, 173-178.

176. Preiser,G., Preiser,L., and Madeo,L. (2003). An outbreak of cryptosporidiosis among veterinary science students who work with calves. J. Am. Coll. Health *51*, 213-215.

177. Pritchard,G.C., Marshall,J.A., Giles,M., Chalmers,R., and Marshall,R.N. (2006). *Cryptosporidium parvum* infection in orphan lambs on a farm open to the public. Vet. Rec. *Submitted*.

178. Puech,M.C., McAnulty,J.M., Lesjak,M., Shaw,N., Heron,L., and Watson,J.M. (2001). A statewide outbreak of cryptosporidiosis in New South Wales associated with swimming at public pools. Epidemiol. Infect. *126*, 389-396.

179. Quiroz,E.S., Bern,C., MacArthur,J.R., Xiao,L., Fletcher,M., Arrowood,M.J., Shay,D.K., Levy,M.E., Glass,R.I., and Lal,A. (2000). An outbreak of cryptosporidiosis linked to a foodhandler. J. Infect. Dis. *181*, 695-700.

180. Rahman,A.S., Sanyal,S.C., Al Mahmud,K.A., and Sobhan,A. (1985). Cryptosporidium diarrhoea in calves & their handlers in Bangladesh. Indian J. Med. Res. *82*, 510-516.

181. Ravn,P., Lundgren,J.D., Kjaeldgaard,P., Holten-Anderson,W., Hojlyng,N., Nielsen,J.O., and Gaub,J. (1991). Nosocomial outbreak of cryptosporidiosis in AIDS patients. BMJ *302*, 277-280.

182. Reduker,D.W. and Speer,C.A. (1985). Factors influencing excystation in Cryptosporidium oocysts from cattle. J. Parasitol. *71*, 112-115.

183. Reduker,D.W., Speer,C.A., and Blixt,J.A. (1985). Ultrastructure of Cryptosporidium parvum oocysts and excysting sporozoites as revealed by high resolution scanning electron microscopy. J. Protozool. *32*, 708-711.

184. Reif,J.S., Wimmer,L., Smith,J.A., Dargatz,D.A., and Cheney,J.M. (1989). Human cryptosporidiosis associated with an epizootic in calves. Am. J. Public Health *79*, 1528-1530.

185. Ribeiro,C.D. and Palmer,S.R. (1986). Family outbreak of cryptosporidiosis. Br. Med. J. (Clin. Res. Ed) *292*, 377.

186. Richardson,A.J., Frankenberg,R.A., Buck,A.C., Selkon,J.B., Colbourne,J.S., Parsons,J.W., and Mayon-White,R.T. (1991). An outbreak of waterborne cryptosporidiosis in Swindon and Oxfordshire. Epidemiol. Infect. *107*, 485-495.

187. Robertson,L.J. and Gjerde,B. (2001a). Factors affecting recovery efficiency in isolation of Cryptosporidium oocysts and Giardia cysts from vegetables for standard method development. J. Food Prot. *64*, 1799-1805.

188. Robertson,L.J. and Gjerde,B. (2001b). Occurrence of parasites on fruits and vegetables in Norway. J. Food Prot. *64*, 1793-1798.

189. Robertson,L.J., Greig,J.D., Gjerde,B., and Fazil,A. (2005). The potential for acquiring cryptosporidiosis or giardiosis from consumption of mung bean sprouts in Norway: a preliminary step-wise risk assessment. Int. J. Food Microbiol. *98*, 291-300.

190. Rodrigues,F., Davies,E.G., Harrison,P., McLauchlin,J., Karani,J., Portmann,B., Jones,A., Veys,P., Mieli-Vergani,G., and Hadzic,N. (2004). Liver disease in children with primary immunodeficiencies. J. Pediatr. *145*, 333-339.

191. Rooney,R.M., Bartram,J.K., Cramer,E.H., Mantha,S., Nichols,G., Suraj,R., and Todd,E.C. (2004a). A review of outbreaks of waterborne disease associated with ships: evidence for risk management. Public Health Rep. *119*, 435-442.

192. Rooney,R.M., Cramer,E.H., Mantha,S., Nichols,G., Bartram,J.K., Farber,J.M., and Benembarek,P.K. (2004b). A review of outbreaks of foodborne disease associated with passenger ships: evidence for risk management. Public Health Rep. *119*, 427-434.

193. Rose,J.B. and Slifko,T.R. (1999). Giardia, Cryptosporidium, and Cyclospora and their impact on foods: a review. J. Food Prot. *62*, 1059-1070.

194. Roy,S.L., DeLong,S.M., Stenzel,S.A., Shiferaw,B., Roberts,J.M., Khalakdina,A., Marcus,R., Segler,S.D., Shah,D.D., Thomas,S., Vugia,D.J., Zansky,S.M., Dietz,V., and Beach,M.J. (2004). Risk factors for sporadic cryptosporidiosis among immunocompetent persons in the United States from 1999 to 2001. J. Clin. Microbiol. *42*, 2944-2951.

195. Rush,B.A., Chapman,P.A., and Ineson,R.W. (1990). A probable waterborne outbreak of cryptosporidiosis in the Sheffield area. J. Med. Microbiol. *32*, 239-242.

196. Sacco,C., Bianchi,M., Lorini,C., Burrini,D., Berchielli,S., and Lanciotti,E. (2006). [Removal of Cryptosporidium and Giardia in drinking water treatment in a Tuscan area]. Ann. Ig *18*, 117-126.

197. Said,B., Wright,F., Nichols,G.L., Reacher,M., and Rutter,M. (2003). Outbreaks of infectious disease associated with private drinking water supplies in England and Wales 1970-2000. Epidemiol. Infect. *130*, 469-479.

198. Sayers,G.M., Dillon,M.C., Connolly,E., Thornton,L., Hyland,E., Loughman,E., O'Mahony,M.A., and Butler,K.M. (1996). Cryptosporidiosis in children who visited an open farm. Commun. Dis. Rep. CDR Rev. *6*, R140-R144.

199. Schets,F.M., Engels,G.B., and Evers,E.G. (2004). Cryptosporidium and Giardia in swimming pools in the Netherlands. J. Water Health *2*, 191-200.

200. Slifko,T.R., Huffman,D.E., Dussert,B., Owens,J.H., Jakubowski,W., Haas,C.N., and Rose,J.B. (2002). Comparison of tissue culture and animal models for assessment of Cryptospridium parvum infection. Exp. Parasitol. *101*, 97-106.

201. Smerdon,W.J., Nichols,T., Chalmers,R.M., Heine,H., and Reacher,M.H. (2003). Foot and mouth disease in livestock and reduced cryptosporidiosis in humans, England and Wales. Emerg. Infect. Dis. *9*, 22-28.

202. Smith,H.V., Girdwood,R.W., Patterson,W.J., Hardie,R., Green,L.A., Benton,C., Tulloch,W., Sharp,J.C., and Forbes,G.I. (1988). Waterborne outbreak of cryptosporidiosis. Lancet *2*, 1484.

203. Smith,H.V., Patterson,W.J., Hardie,R., Greene,L.A., Benton,C., Tulloch,W., Gilmour,R.A., Girdwood,R.W., Sharp,J.C., and Forbes,G.I. (1989). An outbreak of waterborne cryptosporidiosis caused by post-treatment contamination. Epidemiol. Infect. *103*, 703-715.

204. Smith,K.E., Stenzel,S.A., Bender,J.B., Wagstrom,E., Soderlund,D., Leano,F.T., Taylor,C.M., Belle-Isle,P.A., and Danila,R. (2004). Outbreaks of enteric infections caused by multiple pathogens associated with calves at a farm day camp. Pediatr. Infect. Dis. J. *23*, 1098-1104.

205. Soave,R. and Ma,P. (1985). Cryptosporidiosis. Traveler's diarrhea in two families. Arch. Intern. Med. *145*, 70-72.

206. Sopwith,W., Osborn,K., Chalmers,R., and Regan,M. (2005). The changing epidemiology of cryptosporidiosis in North West England. Epidemiol. Infect. *133*, 785-793.

207. Sorvillo,F.J., Fujioka,K., Nahlen,B., Tormey,M.P., Kebabjian,R., and Mascola,L. (1992). Swimming-associated cryptosporidiosis. Am. J. Public Health *82*, 742-744.

208. Spano,F. and Crisanti,A. (2000). Cryptosporidium parvum: the many secrets of a small genome. Int. J. Parasitol. *30*, 553-565.

209. Spano,F., Putignani,L., McLauchlin,J., Casemore,D.P., and Crisanti,A. (1997). PCR-RFLP analysis of the Cryptosporidium oocyst wall protein (COWP) gene

discriminates between C. wrairi and C. parvum, and between C. parvum isolates of human and animal origin. FEMS Microbiol. Lett. *150*, 209-217.

210. Squier,C., Yu,V.L., and Stout,J.E. (2000). Waterborne Nosocomial Infections. Curr. Infect. Dis. Rep. *2*, 490-496.

211. Stafford,R., Neville,G., Towner,C., and McCall,B. (2000). A community outbreak of Cryptosporidium infection associated with a swimming pool complex. Commun. Dis. Intell. *24*, 236-239.

212. Stantic-Pavlinic,M., Xiao,L., Glaberman,S., Lal,A.A., Orazen,T., Rataj-Verglez,A., Logar,J., and Berce,I. (2003). Cryptosporidiosis associated with animal contacts. Wien. Klin. Wochenschr. *115*, 125-127.

213. Stefanogiannis,N., McLean,M., and Van Mil,H. (2001). Outbreak of cryptosporidiosis linked with a farm event. N. Z. Med. J. *114*, 519-521.

214. Sterling,C.R., Seegar,K., and Sinclair,N.A. (1986). Cryptosporidium as a causative agent of traveler's diarrhea. J. Infect. Dis. *153*, 380-381.

215. Strachan,N.J., Ogden,I.D., Smith-Palmer,A., and Jones,K. (2003). Foot and mouth epidemic reduces cases of human cryptosporidiosis in Scotland. J. Infect. Dis. *188*, 783-786.

216. Strohmeyer,R.A., Morley,P.S., Hyatt,D.R., Dargatz,D.A., Scorza,A.V., and Lappin,M.R. (2006). Evaluation of bacterial and protozoal contamination of commercially available raw meat diets for dogs. J. Am. Vet. Med. Assoc. *228*, 537-542.

217. Sturdee,A.P., Bodley-Tickell,A.T., Archer,A., and Chalmers,R.M. (2003). Long-term study of Cryptosporidium prevalence on a lowland farm in the United Kingdom. Vet. Parasitol. *116*, 97-113.

218. Sundkvist,T., Dryden,M., Gabb,R., Soltanpoor,N., Casemore,D., and Stuart,J. (1997). Outbreak of cryptosporidiosis associated with a swimming pool in Andover. Commun. Dis. Rep. CDR Rev. *7*, R190-R192.

219. Sutthikornchai,C., Jantanavivat,C., Thongrungkiat,S., Harnroongroj,T., and Sukthana,Y. (2005). Protozoal contamination of water used in Thai frozen food industry. Southeast Asian J. Trop. Med. Public Health *36 Suppl 4*, 41-45.

220. Szostakowska,B., Kruminis-Lozowska,W., Racewicz,M., Knight,R., Tamang,L., Myjak,P., and Graczyk,T.K. (2004). Cryptosporidium parvum and Giardia lamblia recovered from flies on a cattle farm and in a landfill. Appl. Environ. Microbiol. *70*, 3742-3744.

221. Teunis,P.F., Chappell,C.L., and Okhuysen,P.C. (2002). Cryptosporidium dose response studies: variation between isolates. Risk Anal. *22*, 175-183.

222. Thurston-Enriquez,J.A., Watt,P., Dowd,S.E., Enriquez,R., Pepper,I.L., and Gerba,C.P. (2002). Detection of protozoan parasites and microsporidia in irrigation waters used for crop production. J. Food Prot. *65*, 378-382.

223. Tillett,H.E., de Louvois,J., and Wall,P.G. (1998). Surveillance of outbreaks of waterborne infectious disease: categorizing levels of evidence. Epidemiol. Infect. *120*, 37-42.

224. Wallis,P.M., Matson,D., Jones,M., and Jamieson,J. (2001). Application of monitoring data for Giardia and Cryptosporidium to boil water advisories. Risk Anal. *21*, 1077-1085.

225. Wilberschied,L. (1995). A swimming-pool-associated outbreak of cryptosporidiosis. Kans. Med. *96*, 67-68.

226. Willocks,L., Crampin,A., Milne,L., Seng,C., Susman,M., Gair,R., Moulsdale,M., Shafi,S., Wall,R., Wiggins,R., and Lightfoot,N. (1998). A large outbreak of cryptosporidiosis associated with a public water supply from a deep chalk borehole. Outbreak Investigation Team. Commun. Dis. Public Health *1*, 239-243.

227. Willocks,L.J., Sufi,F., Wall,R., Seng,C., and Swan,A.V. (2000). Compliance with advice to boil drinking water during an outbreak of cryptosporidiosis. Outbreak Investigation Team. Commun. Dis. Public Health *3*, 137-138.

228. Xiao,L., Fayer,R., Ryan,U., and Upton,S.J. (2004). Cryptosporidium taxonomy: recent advances and implications for public health. Clin. Microbiol. Rev. *17*, 72-97.

229. Xu,P., Widmer,G., Wang,Y., Ozaki,L.S., Alves,J.M., Serrano,M.G., Puiu,D., Manque,P., Akiyoshi,D., Mackey,A.J., Pearson,W.R., Dear,P.H., Bankier,A.T., Peterson,D.L., Abrahamsen,M.S., Kapur,V., Tzipori,S., and Buck,G.A. (2004). The genome of Cryptosporidium hominis. Nature *431*, 1107-1112.

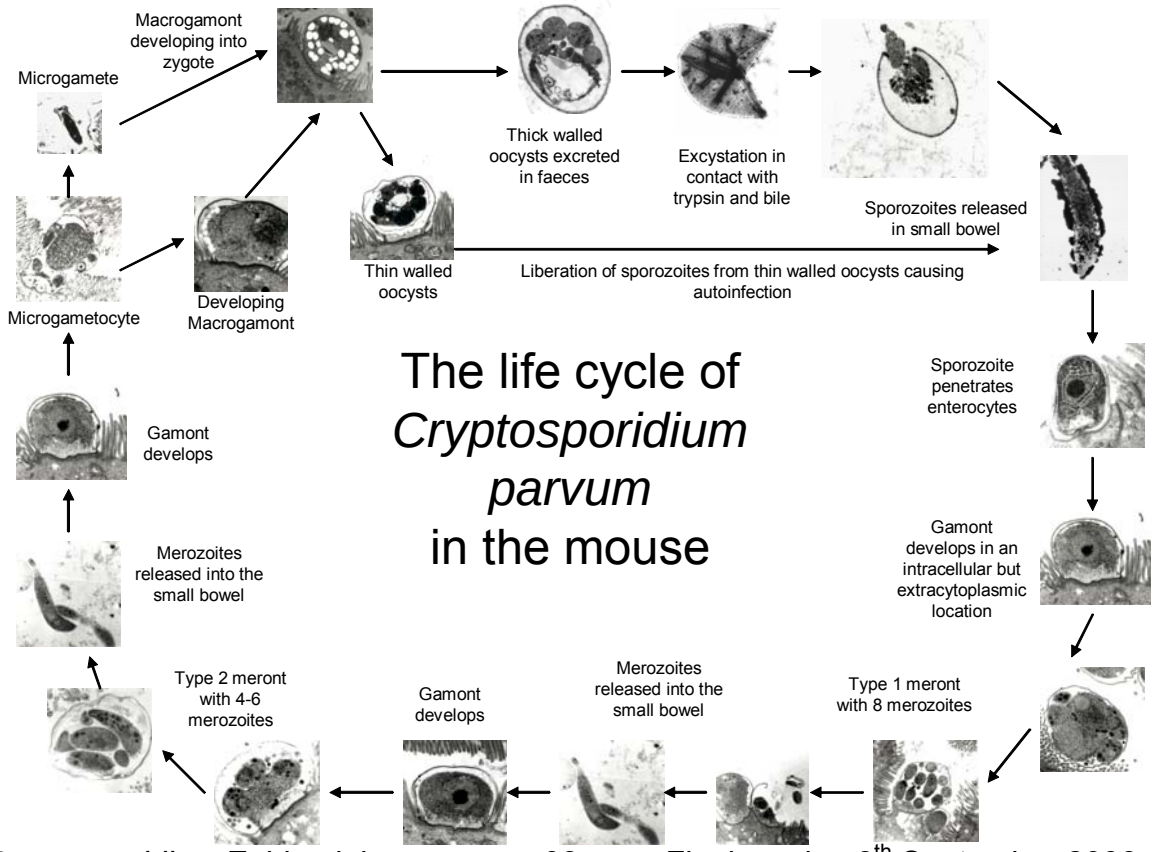
230. Zhou,L., Fayer,R., Trout,J.M., Ryan,U.M., Schaefer,F.W., III, and Xiao,L. (2004). Genotypes of Cryptosporidium species infecting fur-bearing mammals differ from those of species infecting humans. Appl. Environ. Microbiol. *70*, 7574-7577.

13 Appendices

Appendix 1. *Cryptosporidium* biology and life cycle

13.1 *Cryptosporidium* species live inside the epithelial cells of enterocytes within the intestinal tract of a variety of vertebrates, but some species can infect the respiratory tract. The life cycle of *Cryptosporidium* is outlined in Figure 39. Infection starts with ingestion of an oocyst containing four sporozoites. Digestive enzymes trigger the release of sporozoites from the oocyst (excystation). Sporozoites enter epithelial cells, taking up a position that is inside the cell (intracellular) but outside the cytoplasm (extra-cytoplasmic). They develop as trophozoites and undergo an asexual multiple budding process (schizogony) to produce a schizont, which releases merozoites. Merozoites invade other epithelial cells, develop into trophozoites and also undergo schizogony to release more merozoites. Merozoites of this second cycle of asexual reproduction infect further epithelial cells but mature into either 'male' (microgamonts) or 'female' (macrogamonts) gametes to begin the sexual part of the life cycle. Microgamonts produces microgametes which, on release, fertilise the macrogamont and produce a zygote. The zygote may follow two different oocysts generating developmental routes. It can either transform into an oocyst by secretion of a thick wall and development of sporozoites, or into a thin walled oocyst containing sporozoites. Thin walled oocysts are able to liberate sporozoites, thus allowing further rounds of asexual reproduction within the host. *Cryptosporidium* is an obligate parasite and cannot grow outside the body except in tissue culture (Slifko et al., 2002).

Figure 39. An electron microscopy study of the life cycle of *Cryptosporidium parvum* in the mouse



The oocyst

13.2 Two types of oocyst have been described (Current and Reese, 1986; Casemore et al., 1985), a thick-walled one which probably represents the main infectious stage, and a thin-walled one causing autoinfection with a restarting of the life cycle. The thick walled oocyst of *Cryptosporidium* is the stage of the life cycle which is resistant to adverse conditions. Oocysts are passed in large numbers in the faeces of infected individuals, and are widely distributed in the environment. The oocysts of all *Cryptosporidium* spp. contain 4 sporozoites. Oocysts can survive within cool waters for periods in excess of one year. They are sensitive to hydrogen peroxide, ammonia, drying, heating and freezing but are resistant to a range of disinfectants.

13.3 Oocyst excystation is the process by which the four sporozoites present in each oocyst emerge. This can happen in response to trypsin and bile salts (particularly sodium taurocholate) in the intestinal tract, or spontaneously (Reduker and Speer, 1985). Excystation involves the indentation of the oocyst wall, which developed into a cleaved suture spanning 1/3 to 1/2 the circumference of the oocyst. A dimple was present at either end of the suture, and sporozoites left the oocysts anterior end first. Excystation does not occur at room temperature or 4oC, and carbon dioxide is not required (Reduker et al., 1985).

13.4 Oocysts are in the region of 5µm diameter and this small size makes their sedimentation difficult to model using Stoke's Law. This means that their settlement in water is minimal, particularly at ambient UK temperatures (Dai and Boll, 2006; Medema et al., 1998).

13.5 The oocyst is the diagnostic target for routine laboratory testing of faeces, water or environmental samples, whether this is by chemical staining or using immunological test kits. Routine diagnostics do not differentiate between *Cryptosporidium* species.

Typing and speciation

13.6 Typing work on *Cryptosporidium* in England and Wales began in the late 1980s using western blotting techniques (Nichols et al., 1991; McLauchlin et al., 1998; Nichols, 1992) and have subsequently been superseded by PCR and sequencing approaches (McLauchlin et al., 2000; Patel et al., 1999; McLauchlin et al., 1999; Patel et al., 1998; McLauchlin et al., 1998; Spano et al., 1997; Nichols and McLauchlin, 2003). These molecular methods predominantly separated strains into Genotype 1 and 2. Molecular methods have also proved useful in increasing the sensitivity of detecting infection, particularly in immuno-compromised patients (Rodrigues et al., 2004; McLauchlin et al., 2003). Typing has proved useful in case-control studies in outbreaks and in studies of sporadic disease (Hunter et al., 2004; Goh et al., 2004). It has been essential in indicating that *C. hominis* is predominantly a human pathogen and is therefore unlikely to derive from agricultural animals whereas *C. parvum* is a zoonotic organism that can be transmitted from both human and animal sources. Improvements in the technology of species / genotype

identification have allowed the identification of a variety of species from humans with disease (Tables 1 & 2) where previously only two genotypes were recognised (Patel et al., 1998).

Cryptosporidium species

13.7 There are currently 13 recognised species of *Cryptosporidium*, which use different animals as hosts (Table 12).

Table 12. *Cryptosporidium* species (Carey et al., 2004; Xiao et al., 2004)

Cryptosporidium species	Predominant host specificity	Other / detail	Primary location of infection	Oocyst size; length x width (µm)
C. andersoni	Cattle	Bactrian camels, sheep	Abomasum	6.6-8.1 x 5.0-6.5
C. baileyi	Birds	chicken, turkeys, ducks, cockatiels, brown quail, ostrich	Bursa of Fabricius, cloaca	6.0-7.5 x 4.8-5.7
C. bovis	Cattle	sheep	Small intestine	4.8-5.4x4.2-4.8
C. canis	Dogs	coyotes, foxes, humans	Small intestine	4.4-5.4 x 4.2-5.2
C. felis	Cats	humans, cattle	Small intestine	3.2-5.1 x 3.0-4.0
C. galli	Birds	finches, chickens, capercaille , pine grosbeaks	Proventriculus	8.0-8.5 x 6.2-6.4
C. meleagridis	Turkeys	Other birds, humans	Small intestine	4.5-6.9 x 4.2-5.3
C. muris	Mice	hamsters, squirrels, Siberian chipmunks, wood mice, bank voles, rock hyrax, Bactrian camels, mountain goats, humans, cynomolgus monkeys	Stomach	6.5-8.7 x 4.6-6.3
C. molnari	Fish		Stomach, small intestine	3.5-4.7 x 4.2-5.0
C. saurophilum	Reptiles	skink, lizards, monitors, iguanas, geckoes, snakes	Stomach, small intestine	4.4-5.6 x 4.2-5.2
C. scophthalmi	Fish		Small intestine	3.7-5.0x3.0-4.7
C. serpentis	Reptiles	Snakes, lizards	Stomach	5.6-6.6 x 4.8-5.6
C. suis	Pigs	Man	Small intestine	4.9-4.4x4.0-4.3
C. wrairi	Guinea pigs		Small intestine	4.8-5.6 x 4.0-5.0
C. parvum	152 mammalian species	mouse, pig, bear, deer, marsupial, monkey, muskrat, skunk, ferret, cattle, sheep, goats	Small intestine	4.4-5.4 x 4.2-5.2
C. hominis	Humans	rhesus monkeys, dugong, lamb, calves	Small intestine	4.4-5.4 x 4.4-5.9

13.8 There are probably a larger number of species that have yet to be identified. A number of species names were used in the past, but genetic analysis has disproved these as valid species. Many of the species contain genotypes that are genetically distinct from each other and have been found in different host animals. Some of these may eventually be designated as species or sub-species. *C. parvum* has a large number of genotypes that have been isolated from a range of animals (Xiao et al., 2004). The *Cryptosporidium* species infecting humans were originally all described as *C. parvum*. Original work demonstrating differences between *C. parvum* isolates (Nichols et al., 1991) was extended to show two main *C. parvum* genotypes (Peng et al., 1997) that have since been renamed as the species *C.*

hominis (Morgan-Ryan et al., 2002) for the Genotype I and *C. parvum* for Genotype II. The taxonomy of *C. parvum* is still not entirely clear as the original description of this species in a mouse is probably not the organism most commonly reported as *C. parvum* today (Xiao et al., 2004).

13.9 Out of over 13,000 faecal samples examined in England and Wales since 1989 the percentage of human cases caused by *C. parvum* and *C. hominis* is approximately equal (Table 13). *C. meleagridis* comprises less than one percent of cases and all the genotypes that are not *C. parvum* or *C. hominis* comprise only 3.6% of the cases. Evidence from the US suggests that genotypes from wildlife represent little public health risk (Zhou et al., 2004) although *C. parvum* in small mammals may contribute to re-infection of sheep and cattle populations (Chalmers et al., 1997a). The evidence suggests that the sources of infection for humans are predominantly human faeces and the faeces of agricultural animal (mostly cattle and sheep).

Table 13. *Cryptosporidium* species/genotypes detected amongst 13,112 human cases in England and Wales 1989–2005 (Anon, 2002;unpublished data) (Chalmers et al., 2002b; Leoni et al., 2006; Anon, 2002)

Cryptosporidium species	No of patients
C. hominis	6,594 (50.29%)
C. parvum	5,981 (45.6%)
C. hominis and *C. parvum*	65 (0.5%)
C. meleagridis	99 (0.8%)
C. felis	22 (0.2%)
C. canis	2 (0.02%)
C. suis	1 (0.01%)
Cervine genotype	6 (0.05%)
Skunk genotype	1 (0.01%)
CZB141 genotype	1 (0.01%)
Novel or undetermined species/genotype (under further investigation)	337 (2.6%)

Population genetics

13.10 *Cryptosporidium* spp. have a life cycle that is partly asexual and partly sexual. The infecting sporozoites are thought to be haploid, as are all other stages except the zygote, where haploid microgamete and haploid macrogamete meet and go through meiosis to form four haploid sporozoites. If there is a genetic difference between loci of the two gametes then it would be expected that an oocyst would contain the two original alleles (or a re-assorted version) in each of two of the sporozoites. An oocyst might therefore be regarded in one way as a diploid organism with its chromosomes segregated into haploid sporozoites. However this may be an oversimplification as infection is likely to be initiated by more than one oocyst and as a consequence the infection may more closely represent a population

of different haploid sporozoites that are likely to exhibit a dominant genotype but may have one or more different minor alleles.

13.11 In discussing the nature of *Cryptosporidium* population genetics it is useful to characterise the type of population that these organisms are. On the one hand the individual species appear to be panmictic, that is composed of populations that freely interbreed. On the other hand in an outbreak a single type could cause many people to be infected resulting in a predominant genotype that is in a minor sense clonal (the organisms have derived from a common ancestor). Indeed, isolates that are grown under experimental conditions may be regarded as clones. A strictly clonal organism is one in which the entire population has derived from a single organism and is genetically more or less homogeneous. *C. parvum* populations appear to be genetically heterogeneous and panmictic whereas *C. hominis* populations studied so far in the UK are less genetically diverse and appear clonal in nature, although they are likely to remain able to recombine with other *C. hominis* isolates.

Genomes and metabolism

13.12 The genomes of *C. parvum* (Abrahamsen et al., 2004) and *C. hominis* (Xu et al., 2004) have been sequenced. *C. parvum* and *C hominis* both have eight chromosomes (Xu et al., 2004; Spano and Crisanti, 2000; Liu et al., 1999; Piper et al., 1998) and the genome size ranges from 10.4 megabases in *C. parvum* (Liu et al., 1999) to 9.2 for *C. hominis* (Xu et al., 2004). *C. parvum* and *C. hominis* lack an apicoplast (an organelle found in most related genera of the Apicomplexa), but genes associated with apical complex organelles are present. Both species have degenerate mitochondria that lack a genome (Abrahamsen et al., 2004; Xu et al., 2004) and contains plant like genes (Huang et al., 2004). Energy metabolism is largely from glycolysis. Both aerobic and anaerobic metabolisms are available, the former requiring an alternative electron transport system in a simplified mitochondrion. Biosynthesis capabilities are limited, explaining an extensive array of transporters. *C. hominis* and *C. parvum* exhibit very similar gene complements, and phenotypic differences between these parasites are thought to be due to subtle sequence divergences from their common ancestor.

Appendix 2. The principles of communicable disease surveillance.

Communicable disease surveillance is a process that allows the detection, analysis, reporting and control of infectious diseases. It allows the early detection of changes in the temporal, geographic and age distribution of new and known diseases that indicate outbreaks of infection, or changes in the pattern of sporadic diseases.

Analysis of routine and enhanced surveillance data can allow the determination of exposure, prevalence, burden, morbidity, mortality, carriage and long-term trends of infectious diseases.

Surveillance can generate information on changes in the type, pathogenicity and drug resistance of the organisms causing animal and human disease. It can monitor the use and coverage of an intervention, any adverse events arising from that intervention and the overall impact of disease control measures including immunization. Monitoring changes in properties such as prevalence, spatial distribution and time distribution of disease-causing hazards including animal diseases, weather and social factors as well as population vulnerability and susceptibility can give some evidence of causal pathways.

The key purpose of surveillance activities is to create intelligence for action by the HPA and partner organisations to protect public health. This will commonly be at the local level closest to the scene of incidents and outbreaks, but in incidents that are more widespread the action may be regional, national or international. In rare instances, a single case may require prompt national/international intervention, e.g. a case of SARS.

Surveillance informs the development of policies to detect new threats and emerging problems, to reduce exposure to a particular hazard or to protect individuals in advance of such exposure. Normally such policies will be developed nationally in the light of trends in disease and the available methods of prevention.

Information from surveillance can create an early picture of the temporal, geographic and population distribution and the epidemiology of new, poorly understood and well understood diseases for informing decision making for public health, health service planning, risk management, research and control priorities. It can inform key disease eradication or control programs and provide information to support the development of guidance for professionals on the clinical management of individual patients, the choice of the appropriate control strategy and the organisation of services to deliver them to those at risk. Within England and Wales it ensures that the UK can make its full contribution to European and International efforts to protect health.

Surveillance should provide information to professionals and the public about the risks to individuals and the general public.

In order to meet these surveillance objectives, it is essential that coverage of laboratory reporting is complete and the information provided is accurate and timely.

Appendix 3. Cryptosporidium related reference Units on non-Colindale Centre for Infections sites

The UK *Cryptosporidium* Reference Unit. The UK *Cryptosporidium* Reference Unit. This unit, with a staff of six and additional clinical consultant microbiologist sessions, provides core reference services throughout the UK and research to support these core services and their development. It is located in Swansea and is managed by the National Public Health Service for Wales. The unit is headed by Rachel Chalmers.

The core services are:

- differential diagnosis and confirmation of referred isolates of *Cryptosporidium*,
- Specialist testing of samples further to the scope of local laboratory diagnoses
- Typing of referred isolates to support outbreak/cluster investigations and for epidemiological surveillance
- Evaluation of new laboratory methods for *Cryptosporidium*
- Provision of positive control and training materials
- Consultation advice on diagnostic methods and management of cryptosporidiosis
- Advice on the investigation, epidemiology, control and prevention of *Cryptosporidium*
- R & D activities in relation to our reference functions.

Current and recent research undertaken by the UK CRU includes:

- Investigation of genetic variation within *Cryptosporidium hominis* for epidemiological purposes
- Evaluation and risk assessment of zoonotic transmission of *Cryptosporidium* (with Veterinary Laboratories Agency, funded by Defra)
- Comparative trial of *Cryptosporidium* genotyping methods (Sydney Catchment Authority and CRC for Water Quality, Australia)
- Investigation of *Cryptosporidium* clinical isolates and analysis with epidemiological data (with University of East Anglia, funded by Drinking Water Inspectorate)
- Establishing the relationship between farm re-stocking and cryptosporidia: the Caldew catchment study (with the Centre for Research into the Environment and Health, University of Wales, Aberystwyth, funded by UK Water Industry Research and DWI)
- Enhanced surveillance and case control study of sporadic cases of cryptosporidiosis and sero- incidence study (with CDSC North West and University of East Anglia funded by DWI and United Utilities)
- Development of a national reference collection of *Cryptosporidium* oocysts and genotyping (with the Scottish Centre for Infection and Environmental Health, Scottish Parasite Diagnostic Laboratory and Wellcome Institute for Molecular Parasitology, Glasgow University, funded by Scottish Executive and DWI)

- Effects of interventions on population seroprevalence of *Cryptosporidium* (North Cumbria Health Authority)
- Time-series investigation of antibody responses to clinical infection with *Cryptosporidium* (PHLS grant)
- Risk factors for exposure to *Cryptosporidium parvum* in farm workers (PHLS grant)

MSc/PhD students have studied within the UK to successful completion:

2005 PhD "The investigation of the public health significance of protozoan parasites in the environment" (with Professor David Kay, Centre for Research into Health and the Environment, University of Wales Aberystwyth and Professor Stephen Palmer) Guy Robinson, University of Wales College of Medicine, Cardiff

2002 MSC "The seroprevalence of anti-*Cryptosporidium* antibodies in several study populations" Sylvia Arrowsmith, MSc, 2002, University of Wales Institute, Cardiff.

Water Microbiology Quality Control Unit. This unit is based in Newcastle and provides Quality assurance schemes for waterborne indicator organisms, Giardia and *Cryptosporidium*.

The Water Virology Laboratory. This laboratory undertakes surveillance and research on waterborne viruses associated with drinking water, recreational waters and sewage. The unit is located in Reading and is headed by Jane Sellwood.

The Food Research Laboratory. This laboratory is linked to Bristol University and undertakes fundamental and applied research into foodborne pathogens. It is partly funded by the Health Protection Agency. The unit is headed by Tom Humphrey.

London School of Hygiene and Tropical Medicine. This institution is linked to the HPA and provides diagnostic and reference work on parasitic diseases. The unit is headed by Peter Chiodini.

Regional Surveillance Unit (West Midlands)/NHS Direct Health Intelligence Unit Syndromic surveillance of gastrointestinal infections, particularly diarrhoea and vomiting, is undertaken by the RSU (West Midlands) using data provided from 22 NHS Direct sites. Duncan Cooper.

HPA Corporate Affairs Division. This office provides secretarial and administrative support to the Gastrointestinal Diseases Programme and contributes to policy and training initiatives.

Appendix 4. Centre for Infections

The Centre for Infections has been recently formed from the merger of the previous Specialist and Reference Microbiology Division and the Communicable Disease Surveillance Centre of the Health Protection Agency. This merger has enabled closer working between microbiology and epidemiology to the benefit of infection control. The Department of Gastrointestinal Diseases (DGD) was formed in 2005 as part of this restructuring. The Department contains the Laboratory of Enteric Pathogens (LEP) and the Food Safety Microbiology Laboratory (FSML) the Environmental and Enteric Diseases Department formed by the merger of the Gastrointestinal Diseases Department and the Environmental Surveillance Unit within the Communicable Disease Surveillance Centre (CDSC) as well as the Enteric, Respiratory & Neurological Virology Laboratory within SRMD. The DGI is the National Reference Centre for England and Wales and provides a wide range of laboratory services and advice for gastrointestinal, food and waterborne pathogens. In addition the Department has an active Research and Development programme most of which is funded by external grants. The Remit of DGI includes the provision of expert advice, provision of high quality reference and specialist laboratory service, contribution to disease prevention, control and microbial epidemiology, environmental health protection and support, emergency preparedness, policy development and quality improvement, public health related research and development, training and education, partnership with a wide range of other organisations, support of HPA as part of the strategy to combat infectious diseases. The Department is headed by Bob Adak in an acting capacity.

The Environmental and Enteric Diseases Department. This department is part of the Department of Gastrointestinal Diseases and deals with the surveillance, epidemiology and control of gastrointestinal diseases, including cryptosporidiosis, and undertakes research into the causes and interventions that might reduce disease and responds to government and other agencies requests for information and support. Members of the Department are involved in surveillance and research on the microbiology of food and water, and the relations of these to foodborne and waterborne disease. EEDD is also involved in the implementation of food and water legislation, outbreak investigation, advice and training. The Environmental Surveillance Unit was established in 1995 to co-ordinate the food, water and environmental microbiology of the Public Health Laboratory Service (now subsumed into the Health Protection Agency). It arose out of the Environmental Services section of PHLS Headquarters that was established in 1992 and merged with Gastrointestinal Diseases Department in 2004 to form the Environmental and Enteric Diseases Department. This department co-ordinates the investigation of outbreaks that extend across regions or are national. The department interacts with the Food Standards Agency, Defra, the Drinking Water Inspectorate, LACORS, the Environment Agency and other national and International agencies in response to outbreaks of Infection. It liaises with Scotland, Wales, Northern Ireland, Holland and other countries in outbreaks extending across boundaries. It has been involved in a number of research

projects on cryptosporidiosis and provides national eurveillance and epidemiology responses to problems with cryptosporidiosis and has been represented on the Bouchier report. Gordon Nichols is responsible for Cryptosporidium surveillance and epidemiology. The department is headed by Bob Adak.

Enternet. This section is within EEDD and provides an international network on Salmonella and E. coli O157. The unit is headed by Noel Gill.

The Food Safety Microbiology Laboratory. The Food-borne Pathogens Reference Unit and the Biotoxins Section provide reference facilities for: *Bacillus* spp. including *Bacillus cereus*, *Clostridium botulinum*, *Clostridium perfringens*, *Clostridium tetani*, *Listeria* spp including *Listeria monocytogenes*, and the detection of staphylococcal toxins, marine biotoxins and toxic phytohaemagglutinins. The unit developed many of the methods used in *Cryptosporidium* work including typing methods and monoclonal antibodies and has been involved in *Giardia* molecular epidemiology typing work. The unit is headed by Jim McLauchlin.

The Water and Environmental Microbiology Reference Unit (WEMRU) is part of FSML and undertakes and assists with the investigation of outbreaks of water borne disease, in particular legionnaires' disease. It provides advice on the microbiological hazards associated with all kinds of water use and the control of these hazards, in particular: all aspects of the control of Legionella pneumophila the cause of legionnaires' disease; the control of microbiological hazards in swimming, hydrotherapy and spa pools; and methods for the sampling and detection of microorganisms in water and the environment. The Unit also provides teaching and training courses on water and environmental matters. The unit is headed by John Lee.

The Food and Environmental Proficiency Testing Unit (FEPTU) provides internationally recognised external quality assessment (EQA) schemes, also known as proficiency testing schemes, to food microbiology laboratories and laboratories that examine environmental waters for Legionellae. Participants include laboratories in the public and private sectors in over 40 countries. The schemes offer a regular series of stable homogeneous simulated samples of known but undisclosed content that challenge everyday laboratory procedures. The samples are prepared as freeze-dried mixtures of micro-organisms or as LENTICULE discs (a novel procedure for preserving micro-organisms developed by the HPA). The unit is headed by Julie Russell.

HPA London Region - London Food Water and Environmental Microbiology Laboratory (LLFWEL) is part of LARS and is located within FSML. The Laboratory and offers comprehensive services for the microbiological examination of foods and their components, dairy products, waters and environmental samples on behalf of HPA London – London Region and serves 51 local authorities within the Greater London area and some authorities in Bedfordshire, Buckinghamshire and Hertfordshire. The unit is headed by Susanne Surman-Lee.

The Enteric, Respiratory and Neurological Virus Laboratory. The Enteric Virus Unit (EVU) is part of ERNVL, and provides diagnostic and reference services for a wide range of enteric viral pathogens and is involved in national and international outbreak investigations. Diagnostic methods and virus characterisation are available, using both classical and molecular methods, for astroviruses, enteroviruses, enteric adenoviruses, noroviruses (previously known as Norwalk-like viruses or small round structured viruses), rotaviruses and sapoviruses (previously known as Sapporo-like viruses). A comprehensive sequence database of characterised norovirus, sapovirus, astrovirus and rotavirus strains, including geograhical and temporal distributions and the genetic diversity of co-circulating strains, has been established in collaboration with the Bioinformatics Unit. The EVU collaborates with HPA, CDSC, NHS and academic staff in the structured surveillance of enteric virus infections and associated outbreaks. The EVU includes the WHO UK Polio Laboratory which undertakes the isolation and characterisation of poliovirus strains, determines poliovirus immunity and collaborates with NIBSC and the WHO on the laboratory aspects of the worldwide poliovirus eradication campaign. Staff of the EVU are principal investigators or collaborators on research projects funded through externally awarded grants from the MRC, NHS Executive, EU and the Wellcome Trust and the active research programme is reflected in publications and presentations at national and international meetings. The EVU collaborates with industry in the evaluation of products for the diagnosis and characterisation of enteric virus infections. The unit is headed by Jim Gray.

Reference Units within the Laboratory of Enteric Pathogens.

Salmonella Reference Unit (SRU). The SRU provides services for identification and typing of Salmonella spp. Serotyping covers all Salmonella spp. and phage typing is provided for selected serotypes. In addition the SRU offers serodiagnosis for infection with S. Typhi, S. Paratyphi A, B and C. Typing reagents are provided to international reference centres with training for staff from these laboratories.

Escherichia, Shigella, Yersinia and Vibrio Reference Unit (ESYVRU). The ESYVRU provides identification and typing services for Escherichia, Shigella, Yersinia and Vibrio species. Serodiagnostic tests are offered for infection with Y. enterocolitica and Y. pseudotuberculosis and E. coli O157. In addition the Unit examines faecal samples from appropriate clinical cases for non – O157 Vero cytotoxin-producing E. coli and other enterovirulent E. coli. The unit is headed by Tom Cheasty.

Antimicrobial Resistance and Molecular Epidemiology Unit (ARME). Within the Unit all strains of Salmonella, Escherichia coli, Shigella, Yersinia and Vibrio spp referred to the Laboratory of Enteric pathogens are screened for resistance to a wide range of clinically-relevant and epidemiologically-important antimicrobial drugs, using standardised methods at defined breakpoints. The Minimal Inhibitory Concentration (MIC) for specific antimicrobials is determined for cases where antibiotic therapy may be critical for patient management. The Unit also provides DNA-based typing for

Salmonella spp., Shigella spp. and V. cholerae in support of epidemiological investigations. The Unit also has an extensive grant-funded research programme targeted at antimicrobial drug resistance and DNA-based typing of bacterial enteric pathogens nationally and internationally. A range of DNA-based typing and fingerprinting techniques are in use, some of which are organism-specific. Methods include: plasmid typing; pulsed-field gel electrophoresis (PFGE); VT gene subtyping; PCR; PCR-Restriction Fragment Length Polymorphism typing (PCR-RFLP); DNA sequence typing. The unit is headed by John Threlfall.

Campylobacter and Helicobacter Reference Unit. This unit was formed in January 2004 by an amalgamation of the staff and facilities of the two previously independent units. The role of the unit is to provided a specialist and reference service for Campylobacter and Helicobacter in particular for the two main human pathogens, C. jejuni and H. pylori. The aim is to provide a fully accredited service for detection, identification, antibiotic susceptibility testing and typing of these bacteria. With the widespread use of molecular methods, it is planned to develop real time approaches to rapid strain identification for Campylobacter that are essential for epidemiological characterisation, and for rapid case cluster recognition and source identification. Timely recognition of case clusters will enable a public health response to establish the source of infection. The unit will continue to explore new approaches to understanding routes of transmission of H. pylori, surveillance of antibiotic resistance and epidemiology of resistance associated mutations, and application of new diagnostic methods. The unit is actively involved in a number of surveillance projects with CDSC as well in as a variety of projects with external funding (FSA, DEFRA, MRC, DWI and Scottish Executive). The unit is headed by Bob Owen.

The Health Care Associated Infections Department. This department is responsible for the surveillance, epidemiology and control of infections acquired in healthcare settings. In the gastrointestinal diseases area this particularly includes Clostridium difficile and Norovirus related disease. The department is headed by Georgia Duckworth.

Appendix 5. Local and Regional Services

The clinical diagnosis of infectious diseases is conducted in diagnostic microbiology laboratories throughout England and Wales. This includes private hospital, commercial, NHS and HPA laboratories. Within these laboratories are medical and scientific microbiologists, and some hospitals also have Consultants in Infectious Diseases that are not as closely attached to laboratories. National reporting of infectious diseases is through these laboratories and surveillance relies on the completeness and timeliness of reporting. A number of former PHLS laboratories have a status as Collaborating Laboratories with an HPA Consultant attached.

The public health laboratories that remain within the Health Protection Agency (HPA) were originally established under the Public Health Laboratory Service to provide microbiological services that could contribute to the reduction of infectious diseases within England and Wales. This included a remit to assist in the investigation of community outbreaks and undertake specialised work, such as research, virology and food and water examinations, that could not be done in most diagnostic laboratories. These laboratories include the collaborating or commissioned laboratories.

The Food, Water and Environmental (FEW) Microbiology Testing Service is provided by an integrated network of twenty six laboratories in England and four in Wales, an interim national FWE Lead Microbiologist supported by an FWE co-ordinator (based at HPA, Colindale) and nine FWE co-ordinators based in the HPA Regions plus one in Wales (Appendix 6). This structure enables an integrated service at local, regional and national levels so that any potential outbreak can be quickly identified and promptly dealt with. The Health Protection Agency (HPA) is responsible for the delivery of this service in England and the NPHS for this service in Wales. T he laboratories provide specialist microbiology services that are essential to support Local Authorities, the Food Standards Agency, NPHS and the Health Protection Agency in carrying out both statutory and public health functions. Each HPA Region is able to provide a complete range of accredited food, dairy, water and environmental tests from one or more the FWE laboratories within the Region. Local and regional microbiological survey data and the results from public health investigations are organised nationally to provide the scientific evidence base for disease control. Some FWE Laboratories are situated within the HPA or NPHS; some are within local NHS Trusts. In addition, staff within the FWE Laboratory provide expert advice and reference services on food, dairy, environmental and waterborne infections to HPA professionals, epidemiologists, environmental health staff, to local and regional health protection teams and to other agencies. FWE Laboratories conduct research and development work and provide in-service training to few, NPHS, FWE and HPA staff as well as training to staff from external stakeholder organisations such as Local Authorities. Although these laboratories are currently described as Food, Water and Environmental laboratories their remit should not be restricted to those diseases transmitted through these routes, but should include all infectious diseases where public health microbiology

and action may be required. These laboratories work closely with local Environmental Health Departments in responding to outbreaks of infectious diseases. The Welsh FWE Laboratories are managed by the National Public Health Service – Wales.

Each region has a person who is responsible for co-ordinating the food, water and environmental work of the laboratories within the Region. They meet three to four times a year with representatives from the Centre for Infections. They are co-ordinated by a national FWE Lead.

Within LARS in England there are public health teams that usually comprise of Consultants in Health Protection (CHP, but some retain the title of CCDC), public health specialists, infection control / health protection nurses, other scientific and administrative staff, local authority staff and locally based microbiologist. In Wales the Health Protection Teams are part of the NPHS. These teams are supported by regional staff, principally in the area of surveillance and major incident response. Many CCDCs / CHP provide the function of Proper Officer and Port Health Medical Officer for the Local Authority. A number of other key staff may be available informally to the team from Trusts and other organisations, for example PCT personnel, veterinary and other specialist staff. The principal roles of the team include disease surveillance, outbreak identification and management and the development of preventative strategies in conjunction with Regional and National Services.

Within each Local Health Protection Unit there will be a clearly identified individual who will undertake the role of Zonal GI Lead. Zonal leads will develop a special interest in gut pathogens and ensure that current issues are reflected within the team. It is anticipated that they will meet on a regular basis with the Regional GI Lead or Programme Co-ordinator. To be effective the Co-ordinators need to engage with a range of colleagues across local and regional services and in particular link with the various Local Authority food liaison groups, thus providing necessary overview and collaboration with this important group.

Appendix 6. HPA related laboratories conducting Food, Water and Environmental work in England and Wales.

EAST

Chelmsford * Norwich +

EAST MIDLANDS

Leicester + Lincoln + Nottingham + (water samples only)

LONDON

London FWE Microbiology Laboratory, Colindale *

NORTH EAST AND YORKSHIRE AND THE HUMBER

Leeds * Newcastle * Hull + Sheffield +

NORTH WEST

Carlisle + Chester + Preston +

SOUTH EAST

Ashford * Brighton * Wessex Environmental Microbiology Services (WEMS) (Southampton) *

SOUTH WEST

Bristol * Exeter + Gloucester + Plymouth + Truro +

WEST MIDLANDS

Birmingham * Coventry + Shrewsbury/Telford +
Stoke on Trent + (West Midlands Food, Water & Environmental Services) Hereford +

WALES

Bangor¥ Cardiff (¥) Carmarthen (¥) Rhyl (¥)

Health Protection Agency Laboratories (*) and NHS Trust Laboratories (+)
National Public Health Service – Wales (¥)

Appendix 7. Other relevant surveillance bodies

Primary care surveillance. There are three research datasets on primary care that can be used for gastrointestinal diseases:

The General Practice Research Database (GPRD) is the world's largest computerised database of anonymised longitudinal medical records from primary care. Currently data are being collected on over 3 million active patients (approx. 9 million total) from almost 400 primary care practices throughout the UK. It is better coded than Q Research. MRC has bought a copy of GPRD. The database is managed by the GPRD Division of the Medicines and Healthcare products Regulatory Agency (MHRA), the UK's medicines and devices regulator, on a non-profit making basis. The data are updated once every two weeks. GPRD data can be made available to customers via a secure online service to the live data called Full Feature GPRD (FF-GPRD), as project-specific raw data-sets or as aggregate tables. It costs about £250,000 for full access.

QRESEARCH is a high quality database of general practice derived data for use in ethical medical research. It contains data from 468 general practices throughout the UK with records for 3.3 million current patients and 4 million past patients. This includes general practices spread throughout England, Wales, Northern Ireland and Scotland covering every Strategic Health Authority Area. It is run at Nottingham University (HPA access via Gillian Smith). It contains more free text than GPRD (The HPA £70,00pa). When it is fully established, QRESEARCH will contain the records of just under 8 million patients, making it one of the largest aggregated databases in the world. It contains socio-economic details of each patient's postcode but does not contain no strong patient identifiers.

RCGP. The RCGP is the smallest is dataset (60 GP surgeries) and has been going since the late 1980s. Access is through Douglas Flemming (Birmingham). The datasets provide no prescribing data.

Appendix 8. Typing methods

Clinical faecal isolates are typed in a multi-step process. To support the national collection of *Cryptosporidium* oocysts and to provide an archive as a resource for further study, an oocyst suspension is first prepared using salt flotation to separate oocysts from faecal particulate matter. The resulting oocyst suspension is stored at +4^{o}C and an aliquot used for *Cryptosporidium* oocyst disruption by heating, lysis and proteinase k digestion and DNA extraction using the Qiagen kit. The DNA is stored at -20^{o}C.Alternatives to this, by extracting DNA direct from faeces, is being proposed to streamline testing but the archive of oocysts would not be maintained.

At the CRU, routine species identification is performed using a technique known as PCR-RFLP. This involves the polymerase chain reaction (PCR) to amplify targeted parts of a specified gene followed by the identification of polymorphisms within the amplified section by application of restriction enzymes which cutting the gene at recognised sites producing fragment lengths characteristic of species/genotypes. These are called restriction fragment length polymorphisms (RFLP) and are identified by loading the products on to an agarose gel, applying a current to separate the fragments according to their molecular weights and visualising the patterns by staining with a dye. Digital imaging of the gels using special software provides a permanent record of the patterns obtained.

Since large numbers of isolates are characterised on a routine basis at the UK CRU, the gene target used for screening all conformed clinical isolates by PCR-RFLP is the *Cryptosporidium* Oocyst Wall Protein gene. This is a cost-effective target since a single PCR and one restriction enzyme provide a result for the vast majority of isolates and a 24 hour turnaround time has been achieved during outbreak investigations.

The small subunit ribosomal (ssu r) RNA (18s) gene is also used in a nested PCR where greater sensitivity or discrimination is required, but this is more expensive, requiring 2 and sometimes 3 restriction enzymes to discriminate some species and the nested PCR process takes longer to perform. The advantage of the ssu rRNA protocol is that the primers amplify all known species and genotypes of *Cryptosporidium*.

Sequence analysis of an 850 bp stretch of ssu rDNA is undertaken where ambiguous results are obtained by PCR-RFLP, for confirmation of unusual isolates and as a QC exercise.

Environmental specimens are always analysed by DNA sequencing ssu rDNA of up to 5 aliquots of extracted DNA to provide the most clear results in these frequently heterogeneous samples. However, this is both time consuming and costly.

Real time PCR is being adopted by the CRU as a resource-economic mass-screening tool for *C. parvum* and *C. hominis* and to better identify isolates containing both these species. PCR-RFLP and DNA sequencing will be maintained.

The investigation of intra-species variation is still relatively new and there has been no formal evaluation of sub-typing methods or schemes. However, an international comparative trial (Chalmers et al., 2005) showed comparable results between DNA sequencing of a part of the gp 60 gene encompassing a microsatellite region, multilocus typing at three microsatellite DNA regions and single strand conformation polymorphism analysis at the ITS-2.

Current techniques have shown relationships between *C. parvum* subtypes and specific microsatellite markers and risk factors (Anon, 2005). However, 90% *C. hominis* isolates were indistinguishable using microsatellite, and other typing tools are being investigated.

Appendix 9. Prevalence of cryptosporidiosis per 100,000 population per year by Strategic Health Authority (all ages)

STRATEGIC HEALTH AUTHORITIES	1990	1991	1992	1993	1994	1995	1996	1997	1998	1999	2000	2001	2002	2003	2004
Trent	7.4	9.0	8.6	6.0	7.4	8.4	5.9	8.0	7.1	9.3	12.1	6.9	7.4	12.0	6.1
Leicestershire, Northamptonshire And Rutland	4.4	8.2	7.8	10.7	13.8	10.7	6.2	9.6	7.6	6.8	11.0	5.6	4.5	11.3	7.2
Norfolk, Suffolk And Cambridgeshire	5.1	7.4	12.5	9.8	7.3	11.1	5.6	8.8	5.4	10.3	11.0	10.4	8.2	12.7	11.7
Bedfordshire And Hertfordshire	2.5	10.4	10.4	10.1	4.8	14.6	4.4	22.5	3.0	3.8	3.6	5.9	5.7	9.8	10.6
Essex	3.5	6.0	4.3	8.1	7.8	12.1	5.0	4.0	6.9	7.8	12.5	8.4	5.5	14.2	16.0
North West London	2.7	7.2	3.7	2.1	2.8	2.0	1.0	1.7	0.5	0.2	0.1	1.3	0.9	1.0	2.0
North Central London	0.9	4.4	4.3	3.8	3.1	5.5	6.1	2.9	1.2	2.5	2.5	3.4	2.1	3.0	2.4
North East London	0.9	4.5	2.0	1.3	0.8	1.3	1.0	1.5	0.9	1.6	0.1	0.1	0.3	1.4	1.6
South East London	3.2	10.6	3.5	3.0	3.3	3.1	1.0	0.8	0.5	1.3	4.9	1.2	1.6	3.4	2.9
South West London	3.9	10.8	4.9	3.6	3.6	7.4	4.1	5.8	7.1	10.8	13.6	8.2	6.0	10.6	5.8
Northumberland, Tyne & Wear	12.9	6.8	4.2	5.6	6.8	4.7	19.9	6.1	4.5	8.3	9.2	7.0	4.7	10.8	4.6
County Durham And Tees Valley	6.6	4.5	6.0	6.1	7.3	4.8	5.4	5.3	3.7	5.9	8.2	3.9	5.3	11.3	5.1
Cumbria And Lancashire	12.2	16.6	30.9	21.8	21.7	23.2	16.4	23.7	20.0	30.1	33.7	10.6	9.1	10.8	6.9
Greater Manchester	12.6	16.5	22.6	16.6	11.5	11.4	12.1	21.9	14.9	28.8	28.0	10.0	11.4	13.8	8.1
Cheshire & Merseyside	3.2	4.5	5.6	4.8	3.5	5.1	4.8	4.2	4.5	5.7	4.5	3.1	4.2	8.2	5.0
Thames Valley	5.4	8.8	7.5	11.7	10.4	13.4	6.3	7.9	9.7	8.6	11.2	8.1	5.6	9.8	4.4
Hampshire And Isle Of Wight	14.0	12.1	4.7	5.6	4.0	5.8	6.3	4.2	4.0	5.5	4.3	4.3	4.1	4.2	1.1
Kent And Medway	7.2	8.7	4.8	6.9	7.2	7.4	3.9	3.0	2.8	4.2	4.8	5.4	4.0	11.4	5.3
Surrey And Sussex	10.6	16.3	9.4	8.4	11.2	13.3	6.2	9.8	6.6	8.7	12.4	8.6	7.5	13.6	8.1
Avon, Gloucestershire And Wiltshire	15.5	12.7	9.6	8.2	10.7	9.6	6.3	7.5	7.4	7.9	10.8	5.7	5.1	17.8	6.8
South West Peninsula	13.3	19.2	24.4	14.5	15.1	47.2	10.9	11.2	12.8	16.8	18.6	11.2	10.2	16.3	12.2
Somerset And Dorset	14.1	15.4	9.2	16.5	13.6	12.2	11.7	14.2	12.2	16.7	17.4	11.1	8.5	15.6	12.9
Shropshire And Staffordshire	11.4	12.7	11.1	12.1	8.6	15.0	10.7	6.7	7.4	12.0	14.1	9.2	7.6	15.9	8.9
Birmingham And The Black Country	4.4	3.5	4.4	4.2	3.7	6.3	6.7	7.5	6.2	8.7	10.1	7.9	4.0	11.3	4.8
Coventry, Warwickshire, Herefordshire And Worcestershire	7.9	5.2	9.3	12.4	3.2	2.6	1.9	2.1	3.0	5.4	7.3	4.9	3.4	9.2	6.0
Wales	6.8	12.1	11.4	12.1	11.6	12.6	8.0	7.3	9.4	11.7	12.5	8.5	6.1	11.0	6.2
North And East Yorkshire And Northern Lincolnshire	43.0	14.2	16.3	11.2	16.5	19.3	8.3	10.3	14.4	12.0	13.5	10.7	6.0	22.2	10.2
West Yorkshire	14.8	11.5	15.7	15.7	15.7	15.0	6.9	8.5	6.2	7.0	6.1	8.0	7.6	15.4	5.8
South Yorkshire	14.9	10.0	11.9	11.4	11.5	14.4	7.9	11.1	6.8	11.5	16.0	7.7	5.4	12.1	10.0

*Two-sample t test with unequal variances was performed on years 1990 to 2000 and 2001 to 2004 to test for significant differences between these year periods for Strategic Health Authority. Differences were significant for Cumbria & Lancashire (p=0.00001) and Greater Manchester (p=0.0086). For other Strategic health Authorities the differences were not significant.

Appendix 10. Prevalence of Cryptosporidium infection in 1-4 year olds in Strategic Health Authorities in England and Wales 1990-2004

Strategic health authority name	1990	1991	1992	1993	1994	1995	1996	1997	1998	1999	2000	2001	2002	2003	2004
Trent StHA	43	53	42	35	31	44	31	44	44	51	51	32	38	43	23
Leicestershire, Northants & Rutland StHA	34	53	22	50	54	48	22	57	47	46	62	26	12	59	38
Norfolk, Suffolk And Cambridgeshire StHA	30	52	62	54	45	47	29	55	35	51	62	69	40	65	59
Bedfordshire And Hertfordshire StHA	10	41	72	53	38	94	27	125	20	30	24	36	26	60	56
Essex StHA	37	64	35	62	58	72	35	23	54	56	76	48	27	74	87
North West London StHA	30	65	35	29	29	18	8	15	6	3	1	3	0	11	24
North Central London StHA	0	3	16	8	6	13	9	9	2	11	13	12	3	13	11
North East London StHA	6	32	13	10	4	7	6	9	4	10	1	0	5	9	6
South East London StHA	22	73	25	24	27	29	7	1	5	7	43	4	8	17	14
South West London StHA	35	83	37	18	31	55	28	42	51	87	90	67	49	73	42
Northumberland, Tyne & Wear StHA	95	50	24	26	34	40	106	48	33	38	58	38	14	76	37
County Durham and Tees Valley StHA	24	30	30	37	38	24	40	26	18	29	39	13	27	64	26
Cumbria And Lancashire StHA	98	137	212	164	153	175	121	169	165	244	185	60	54	68	40
Greater Manchester StHA	88	112	118	93	54	64	69	121	97	149	139	35	47	65	54
Cheshire & Merseyside StHA	23	36	45	33	27	32	31	33	30	42	24	22	26	48	36
Thames Valley StHA	46	56	43	90	75	99	42	71	82	60	54	41	29	52	27
Hampshire And Isle Of Wight StHA	105	106	36	61	44	66	67	42	39	68	43	45	40	58	12
Kent And Medway StHA	39	48	19	32	37	35	24	20	15	27	19	22	19	55	19
Surrey And Sussex StHA	101	137	79	68	93	102	53	79	56	75	115	57	66	85	64
Avon, Gloucestershire And Wiltshire StHA	130	113	86	75	89	80	48	77	51	67	75	51	36	107	48
South West Peninsula StHA	155	206	215	161	143	417	121	101	121	183	195	95	103	150	95
Dorset And Somerset StHA	135	159	86	125	109	83	102	99	123	138	168	72	64	102	94
Shropshire And Staffordshire StHA	73	99	64	73	53	75	77	37	51	60	54	61	45	63	34
Birmingham And The Black Country StHA	21	18	26	16	16	30	27	36	34	35	28	35	19	48	23
West Midlands South STHA	57	31	86	99	19	17	11	11	23	58	39	30	27	44	45
Wales	55	93	91	97	97	94	77	63	92	103	102	64	55	81	53
N and E Yorkshire And N Lincs StHA	40	23	19	42	67	64	23	39	58	35	39	26	14	66	34
West Yorkshire StHA	62	79	68	109	141	111	46	60	58	63	42	74	77	128	52
South Yorkshire StHA	4	20	19	36	22	32	16	20	17	25	40	14	13	27	35
Percentage of StHAs with more than the average prevalence in this year	44.8	72.4	51.7	72.4	44.8	69	17.2	41.4	34.5	62.1	58.6	17.2	0	75.9	27.6

*Two-sample t test with unequal variances was performed on years 1990 to 2000 and 2001 to 2004 to test for significant differences between these year periods for Strategic Health Authority. Differences were significant for Cumbria & Lancashire (p=0.00001), Greater Manchester (p=0.0007), Thames Valley (p=0.0008), South West Peninsula (p=0.03), Dorset & Somerset (p=0.01), and Wales (0.02). For other Strategic health Authorities the differences were not significant.

Appendix 11. Age distribution of cryptosporidiosis in Strategic Health Authorities in England and Wales 1989-2005

Strategic health authority name	0	1-4	5-9	10-14	15-19	20+	Other	Grand Total	% <5 years
AVON, GLOUCESTERSHIRE AND WILTSHIRE StHA	222	1530	615	267	137	1124	69	3964	44.2
BEDFORDSHIRE AND HERTFORDSHIRE StHA	98	663	310	140	62	683	130	2086	36.5
BIRMINGHAM AND THE BLACK COUNTRY StHA	80	623	379	249	109	946	176	2562	27.4
BRO TAF	43	336	214	124	46	322	76	1161	32.6
CHESHIRE & MERSEYSIDE StHA	94	669	390	173	62	541	46	1975	38.6
COUNTY DURHAM AND TEES VALLEY StHA	53	308	215	114	55	384	41	1170	30.9
CUMBRIA AND LANCASHIRE StHA	243	2162	998	424	211	1753	279	6070	39.6
DORSET AND SOMERSET StHA	95	1017	465	233	150	837	58	2855	38.9
DYFED POWYS	82	542	157	64	18	121	52	1036	60.2
ESSEX StHA	105	744	459	206	63	605	33	2215	38.3
GREATER MANCHESTER StHA	271	2037	935	458	274	2230	652	6857	33.7
GWENT	10	210	68	31	12	88	2	421	52.3
HAMPSHIRE AND ISLE OF WIGHT StHA	205	911	226	80	58	424	29	1933	57.7
IECHYD MORGANNWG	12	96	97	37	8	55	5	310	34.8
KENT AND MEDWAY StHA	58	446	265	147	70	771	46	1803	28.0
LEICESTERSHIRE, NORTHANTS & RUTLAND StHA	72	550	315	174	102	773	153	2139	29.1
N AND E YORKSHIRE AND N LINCS StHA	154	1212	644	339	170	1464	120	4103	33.3
NORFOLK, SUFFOLK AND CAMBRIDGESHIRE StHA	88	876	564	276	156	1234	118	3312	29.1
NORTH CENTRAL LONDON StHA	30	89	35	19	2	149	288	612	19.4
NORTH EAST LONDON StHA	26	116	57	21	8	66	19	313	45.4
NORTH WALES	124	841	426	173	86	593	29	2272	42.5
NORTH WEST LONDON StHA	62	309	40	7	7	204	62	691	53.7
NORTHUMBERLAND, TYNE & WEAR StHA	73	534	346	181	81	545	51	1811	33.5
SHROPSHIRE AND STAFFORDSHIRE StHA	101	789	425	237	129	1059	106	2846	31.3
SOUTH EAST LONDON StHA	67	369	122	45	14	265	40	922	47.3
SOUTH WEST LONDON StHA	124	701	256	102	32	392	151	1758	46.9
SOUTH WEST PENINSULA StHA	221	1914	743	343	149	957	121	4448	48.0
SOUTH YORKSHIRE StHA	88	543	393	198	107	1062	82	2473	25.5
SURREY AND SUSSEX StHA	307	1747	734	232	113	1341	107	4581	44.8
THAMES VALLEY StHA	170	1133	510	196	131	872	222	3234	40.3
TRENT StHA	132	869	525	301	149	1534	99	3609	27.7
WEST MIDLANDS SOUTH STHA	104	567	254	99	37	301	305	1667	40.3
WEST YORKSHIRE StHA	190	1267	744	355	124	1045	175	3900	37.4
Grand Total	3804	26720	12926	6045	2932	24740	3942	81109	37.6

Appendix 12. Cases of *Cryptosporidium* infection by year and month

North West

Earliest specimen year	01	02	03	04	05	06	07	08	09	10	11	12	Grand Total
1989	35	35	50	66	79	85	99	151	176	113	127	57	1073
1990	46	35	36	36	46	42	69	88	92	60	35	43	628
1991	39	42	63	100	86	45	47	60	123	89	99	48	841
1992	49	34	89	128	226	168	109	76	126	117	90	83	1295
1993	50	43	55	95	110	138	133	91	99	47	47	43	951
1994	64	32	60	110	100	74	41	60	90	76	54	28	789
1995	43	31	48	55	69	53	40	101	128	72	124	90	854
1996	60	46	61	73	91	55	51	54	89	53	52	48	733
1997	24	21	86	85	183	260	71	63	70	76	98	65	1102
1998	52	27	57	148	138	96	56	49	71	72	56	42	864
1999	31	58	178	122	321	159	91	92	137	103	71	66	1429
2000	55	42	43	62	330	170	68	127	158	194	145	57	1451
2001	34	28	37	38	20	12	18	35	74	88	84	58	526
2002	34	30	49	47	56	56	45	44	37	53	77	31	559
2003	23	23	27	23	26	26	90	128	221	83	48	31	749
2004	25	21	36	43	41	14	24	48	88	63	40	14	457
Grand Total	664	548	975	1231	1922	1453	1052	1267	1779	1359	1247	804	14301

Other regions

Earliest specimen year	01	02	03	04	05	06	07	08	09	10	11	12	Grand Total
1989	289	564	969	696	593	387	433	690	791	657	580	310	6959
1990	549	444	413	322	320	261	271	361	416	344	298	280	4279
1991	384	275	455	584	414	277	314	350	529	405	307	291	4585
1992	182	184	237	289	288	282	238	353	561	484	549	335	3982
1993	226	236	323	581	479	382	335	294	387	299	290	220	4052
1994	150	141	283	468	362	338	211	332	547	333	427	259	3851
1995	197	192	232	321	343	181	205	647	1008	987	441	213	4967
1996	318	255	249	289	292	213	198	268	335	240	186	144	2987
1997	102	189	382	351	266	225	242	276	441	357	312	223	3366
1998	150	108	166	382	257	191	206	253	389	272	291	157	2822
1999	97	76	210	358	255	212	198	328	741	474	460	214	3623
2000	106	98	211	228	405	230	256	522	837	727	509	253	4382
2001	144	129	116	190	158	126	139	347	703	526	418	214	3210
2002	142	124	119	232	178	173	150	232	385	310	298	261	2604
2003	133	144	209	183	238	191	466	907	1489	639	370	347	5316
2004	176	148	212	297	266	161	193	380	569	363	378	157	3300
Grand Total	3345	3307	4786	5771	5114	3830	4055	6540	10128	7417	6114	3878	64285

Appendix 12 (continued). Cases of *Cryptosporidium* infection by year and month

Total cases

Specimen year	01	02	03	04	05	06	07	08	09	10	11	12	Total
1989	324	599	1019	762	672	472	532	841	967	770	707	367	8032
1990	595	479	449	358	366	303	340	449	508	404	333	323	4907
1991	423	317	518	684	500	322	361	410	652	494	406	339	5426
1992	231	218	326	417	514	450	347	429	687	601	639	418	5277
1993	276	279	378	676	589	520	468	385	486	346	337	263	5003
1994	214	173	343	578	462	412	252	392	637	409	481	287	4640
1995	240	223	280	376	412	234	245	748	1136	1059	565	303	5821
1996	378	301	310	362	383	268	249	322	424	293	238	192	3720
1997	126	210	468	436	449	485	313	339	511	433	410	288	4468
1998	202	135	223	530	395	287	262	302	460	344	347	199	3686
1999	128	134	388	480	576	371	289	420	878	577	531	280	5052
2000	161	140	254	290	735	400	324	649	995	921	654	310	5833
2001	178	157	153	228	178	138	157	382	777	614	502	272	3736
2002	176	154	168	279	234	229	195	276	422	363	375	292	3163
2003	156	167	236	206	264	217	556	1035	1710	722	418	378	6065
2004	201	169	248	340	307	175	217	428	657	426	418	171	3757
Total	4009	3855	5761	7002	7036	5283	5107	7807	11907	8776	7361	4682	78586
Average 1989 to 2000	275	267	413	496	504	377	332	474	695	554	471	297	5155
Average 1989 to 1992	393	403	578	555	513	387	395	532	704	567	521	362	5911
Average 1993 to 1996	277	244	328	498	462	359	304	462	671	527	405	261	4796
Average 1997 to 2000	154	155	333	434	539	386	297	428	711	569	486	269	4760
Average 2001 to 2004	178	162	201	263	246	190	281	530	892	531	428	278	4180
p value in T test NW Region*	0.0034	0.0058	0.183	0.0003	0.002	0.0017	0.1828	0.4271	0.8531	0.2361	0.1953	0.0848	
p value in T test Others regions*	0.0687	0.0465	0.192	0.0027	0.0031	0.0011	0.7995	0.6513	0.4678	0.9577	0.6292	0.9454	
p value in T test Total*	0.0339	0.0273	0.0061	0.0007	0.0001	0.0001	0.6298	0.7697	0.5432	0.8381	0.4162	0.6992	

*Two-sample t test with unequal variances was performed on years 1989-2000 and 2001 to 2004 to test for significant differences between these year periods for each month. There was a significant difference for the North West region and for other regions for January, February, April, May and June. For all regions there was a significant difference for January, February, March, April, May and June. There were no significant reductions in cases the second half of the year.

Appendix 13. Developing a model of cryptosporidiosis 1989 - 1999

The weekly totals of laboratory confirmed cases of cryptosporidiosis reported to national surveillance between 1989 and 1999 were obtained for England and Wales. For each case the date was based upon when the stool specimen was taken. Cases where the individual had reported recent foreign travel (4.6%) were removed from the main dataset but kept separately as a potential measure of imported infections.

Weekly temperatures were obtained from the Central England Temperature series and precipitation from the UK Meteorological Office. An estimate of national weekly river flow was derived from the maximum weekly flow for each of the three major rivers in England and Wales: the Thames, the Severn and the Trent. These values were averaged to produce one national estimate. To account for lagged relationships with precipitation, temperature or river flow, variables were constructed indicating the values for each of the twelve previous weeks.

The association between cryptosporidiosis and the environmental variables may vary at different times of the year. Consequently, the data were analysed by taking each week in turn and using Ordinary least-squares regression to examine the relationship between the cryptosporidiosis cases, the weather variables and numbers of travel cases that week. Results for individual weeks were then combined based upon those with similar seasonal relationships. This produced three time periods, mid March to the end of June (weeks 11 – 26), July to early September (weeks 27 – 36) and early September to mid March (weeks 37 – 10).

When the data from individual weeks were combined it became important to control for seasonality and so all models were analysed incorporating dummy variables for each of the individual weeks. Due to the fact that cryptosporidiosis is an infection the number of cryptosporidiosis cases in the previous week may have an impact upon the number of cases in the current week. Therefore all models included a variable indicating the number of cryptosporidiosis cases in the previous week. We also included two variables indicating the number of public holidays in the current and previous week. These were to control for reporting artefacts in the data. Further details of the methods and variables are published in the literature(3).

Results 1989 - 1999

The results of the Ordinary least-squares regression demonstrated that between mid March and the end of June (weeks 11 – 26) the weekly cryptosporidiosis cases were strongly associated with the maximum river flow two weeks before. Higher river flows were associated with more cryptosporidiosis cases. This is presented in Table 14. During this period cryptosporidiosis may be driven by the release of large numbers of newborn, and hence highly infectious, animals onto the land. At this time the land is usually saturated with water so any excrement and its accompanying *Cryptosporidium* are readily washed into watercourses, hence the strong link to river flow.

Table 14: Ordinary least-squares regression model of weekly cryptosporidiosis cases between mid-March and the end of June (Weeks 11 – 26)

Variable[1]	Estimate[2]
Number of cryptosporidiosis cases in the previous week	0.731*
Number of bank holidays the current week	-23.96*
Number of bank holidays the previous week	25.76*
Maximum river flow two weeks before (m^3/sec)	0.0896*
Intercept	9.219

r^2 79.25%, number of observations = 176, * p<0.05

[1] The model also included 17 presence / absence variables indicating to which week each observation was a member

[2] The change in cases in weekly cryptosporidiosis cases per unit change in the explanatory variable

Between July and early September Table 15 indicates that cryptosporidiosis is significantly associated with rainfall between five and eight weeks in the past and temperature between 1 and 4 weeks in the past. Together these suggest that the cryptosporidiosis is higher if the previous weather is warm and relatively dry. Warm dry weather is associated with times of high soil moisture deficit when any *Cryptosporidium* falling onto the land is likely to build up and not be transferred to water courses. The association with the previous four weeks temperature may also be indicative of periods when outdoor activities such as swimming and countryside visits are common. Between early September and mid-March no weather or travel variables were significant in the model which is presented in Table 16.

Table 15: Ordinary least-squares regression model of weekly cryptosporidiosis cases between July and early September (weeks 27 – 36)

Variable[1]	Estimate[2]
Cryptosporidiosis cases the previous week	0.9933
Number of bank holidays the current week	-24.22*
Number of bank holidays the previous week	17.56
Average rainfall between the previous 5 and 8 weeks	-.5827*
Average temperature in the previous 1 to 4 weeks	.3077*
Intercept	37.02*

r^2 88.72%, numbers of observations = 110, * p< 0.05

[1] The model also included 10 presence / absence variables indicating to which week each observation was a member

[2] The change in cases in weekly cryptosporidiosis cases per unit change in the explanatory variable

Table 16: Ordinary least-squares regression model of weekly cryptosporidiosis rate between early September and mid-March (weeks 37 – 10)

Variable[1]	Estimate[2]
Cryptosporidiosis cases the previous week	.6844*
Number of bank holidays the current week	-6.308
Number of bank holidays the previous week	19.26
Intercept	1.868

r^2 59.15% , number of observations = , * p< 0.05

[1] The model also included 26 presence / absence variables indicating to which week each observation was a member

[2] The change in cases in weekly cryptosporidiosis cases per unit change in the explanatory variable

This section has developed predictive models of weekly cryptosporidiosis cases. All the models had high explanatory power, were biologically plausible and consistent with previous research (3). These models were then used to predict the number of cryptosporidiosis cases post 2000 that would have been expected had the new regulations not been implemented.

Appendix 14. Outbreaks of *Cryptosporidium* involving public water supplies in the UK: 1983-2005

Reference/CDSC code	Region	Date	No. of cases in outbreak (lab positive)	Hospital Cases	Pathogen in Water	Faecal Indicator Organisms in water	Analytical Epidemiology Association	Descriptive Epidemiology Association	Additional Information	Strength of Association
Galbraith (1987)	South East	1983	16	NR	No	NR	-	Yes	The geographic distribution of cases linked the outbreak to a public water supply.	Possible
Galbraith (1987)	South East	1985	50	NR	No	NR	-	Yes	The geographic distribution of cases matched a public water supply. A period of heavy rainfall preceded the outbreak.	Possible
Rush et al (1990)	Yorkshire & Humber	May-Jun 86	62	NR	No	NR	Yes	Yes	Case-case study showed association between cryptosporidiosis and public supply from one reservoir. Oocysts detected in river/streams and untreated samples. Cattle near reservoir were possible source of contamination.	Probable
Smith et al (1989)	Scotland	Apr-88	27 (27)	NR	Yes	No	-	Yes	All cases drank from the affected supply. Pipe collecting runoff discharged into post-treatment storage tank. Faecal indicators were detected in break-pressure tank but not in treated water..Cattle slurry spraying and heavy rainfall occurred in the area prior to the outbreak.	Strong
Richardson et al (1991)	South West	Jan-89	516 (516)	41	Yes	NR	-	Yes	Mapping of cases suggested an association between cryptosporidiosis and surface water supply from Thames. Higher attack rates occurred in areas supplied by the affected water supply.	Strong
Barer & Wright (1990)	Scotland	89/90	442	NR	NR	NR	-	Yes	One third of cases received their drinking water supply from Loch Lomond. The remainder had contact with other cases or farm animals or had travelled abroad. Oocysts were detected in the Loch.	Possible/ Probable
CDSC records	South East	Feb-89	65 (65)	NR	Yes	NR	-	Yes	Descriptive epidemiology was suggestive of association between illness and consumption of un-boiled mains water.	Strong
CDSC records/ Aston et al (1991)	Yorkshire and Humber	Dec-89	500 (477)	NR	No	NR	Yes	NR	Case-control study showed an association between illness and the consumption of un-boiled mains water, with a dose-response effect. Slow sand filters at treatment works had been by-passed prior to illness in community.	Strong
CDSC records	North East	Aug-90	300 (5)	NR	No	Yes	Yes	NR	Mixed outbreak of Campylobacter and *Cryptosporidium.* Cohort study showed an association between illness and consumption of un-boiled mains water.	Strong

Joseph et al (1991)	South East	Dec-90 – Jan-91	47 (47)	NR	No	NR	Yes	Yes	Case control study showed a dose-response association between illness and consumption of un-boiled tap water. Treated river water had been used to supplement borehole water.	Probable
Maguire et al (1995)	London	Jan-Feb 91	44 (44)	NR	No	NR	Yes	NR	Case control study showed an association between illness and consumption of >1 glass/day mains water from one company. The size of the outbreak may have been underestimated due to inconsistent London-wide *Cryptosporidium* screening at that time. History of oocysts in implicated public supply.	Probable
Bouchier (2001)	UK	Apr-91	5	NR	No	NR	NR	NR	The affected supply was derived from crudely filtered spring, well and stream water. Agricultural contamination of the well was suspected.	Possible
CDSC records	South East	Oct-91	15 (15)	NR	Yes	NR	-	Yes	-	Strong
92/072	North West	Mar-92	18	NR	NR	No	No	Yes	Case-control study did not show any association between illness and water exposure. Cases were geographically linked to one water supply. Faecal indicators were detected in this supply in January 1992 (one sample).	Possible
Irvine (2001)/ Bouchier (2001)	Scotland	Apr-92	50	NR	Yes	NR	NR	NR	The outbreak is likely to have been associated with Loch Lomond. Potential sources include contamination from sewage treatment works/farm drains and grazing animals.	Probable
92/133	North West	Apr-June 92	77 (77)	8	No	No	Yes	Yes	This outbreak occurred in multiple locations in the region. Case control study showed an association between illness and consumption from a surface-water derived mains supply. During this time water treatment works under construction drained downstream of the lake.	Probable
92/327 Vincent (1997)	South West	Jun-Nov 92	204 (204)	NR	No	No	Yes	Yes	Case-control study confirmed an association between illness and consumption of mains water. There was a slight increase in rainfall, river flow and turbidity prior to the outbreak.	Probable
92/249 Atherton et al (1995)	Yorkshire & Humber	Oct-Dec 92	125 (125)	9	Yes	NR	Yes	Yes	Case control study confirmed an association between illness and drinking from the suspected supply. Water leaving treatment plant showed increased turbidity, following heavy rainfall.	Strong
92/287 Bridgman et al (1995)	North West	Nov92 - Feb 93	47 (47)	5	No	No	Yes	Yes	Case-control study confirmed an association between illness and drinking un-boiled water from two groundwater supplies. A dose-response relationship was demonstrated. One unfiltered groundwater supply may have become contaminated by septic tank drainage close to the borehole. Heavy rainfall and increased turbidity in the distribution reservoir preceded the outbreak.	Strong
Bouchier (2001)	UK	Apr-93	Approx. 3	NR	Yes	NR	NR	NR	This outbreak was linked to an unfiltered supply. Animal contamination of the stream source may have occurred..	Probable

Bouchier (2001)	UK	Apr-93	Approx. 48	NR	No	NR	NR	NR	The suspected unfiltered supply derived from an upland reservoir. Possible run-off from fields with grazing animals after heavy rainfall was a potential source of contamination.	Possible/ Probable
Irvine (2001)	Scotland	Apr-Jun 93	158	NR	NR	NR	-	Yes	The majority of cases occurred in one water distribution zone. This supply was drawn from an upland reservoir, surrounded by cattle.	Possible
93/105 Morgan et al (1995)	South West	Apr-May 93	64 (40)	4	Yes	No	Yes	Yes	Case control study showed a dose-response relationship between consumption of un-boiled tap water from a partially filtered borehole supply and illness. In May/June, low oocyst concentrations were detected in the reservoir serving the town. Subsequent inspection showed no major structural defects in the supply's service reservoirs.	Probable
93/330	Yorkshire & Humber	Jun-July 93	97 (97)	1	No	No	Yes	Yes	Case control study showed an association between illness & consumption of un-boiled tap water. Chlorination failure and excessive head on filters were identified.	Probable
93/227	South East	July-Oct 93	27 (27)	0	No	No	Yes	Yes	Case control study showed consumption of un-boiled tap water and visiting leisure centre as risk factors for illness.	Probable
Bouchier (2001)	UK	Jun-94	8	NR	No	NR	NR	NR	The suspected supply was drawn from a natural reservoir fed by a spring. Animal faecal contamination may have occurred following heavy rainfall.	Possible/ Probable
94/349 CDSC (1995)	South East	Aug-Dec 94	229 (229)	5	Yes	No	Yes	Yes	Case control study confirmed an association between illness and consumption of mains water.	Strong
94/395 CDSC (1995)	East Midlands	Oct-94	33 (33)	0	No	No	-	Yes	Cases were geographically linked to a single public water supply.	Possible
Bouchier (2001)	UK	Feb-95	40	NR	No	Yes	NR	NR	This outbreak was linked to an unfiltered spring water supply contaminated with animal faecal matter after heavy rainfall.	Possible/ Probable
95/685 CDSC (1996a)	South West	Jul-Sept 95	575 (575)	25	Yes	No	Yes	Yes	Cohort study confirmed an association between illness and consumption of water from the implicated supply. Factors contributing to the outbreak were ingress of surface water into groundwater, inadequate filtration as demand exceeded capacity and inadequate monitoring. Heavy rainfall had also followed a period of drought.	Strong
96/006 CDSC (1996b)	North East	Jan-Feb 96	126 (126)	6	No	Yes	Yes	Yes	Storms and upstream agricultural pollution placed excess burden on the water treatment plant. Case control study confirmed an association between illness and consumption of un-boiled tap water.	Strong
96/164 CDSC (1996b)	Yorkshire and Humber	Feb-Mar 96	20 (20)	3	No	No	-	Yes	Algal bloom resulted in increased turbidity in the storage tank prior to entering the treatment plant. Oocysts were detected in untreated water but not treated water samples.	Probable

96/275 CDSC (1996b)	North West	Mar-May 96	107 (107)	NR	Yes	No	Inconclusive	Yes	Case control study was inconclusive. There was no evidence of treatment failure or excess strain on the water treatment plant. A low concentration of oocysts was detected in treated water.	Strong
97/271 CDSC (1997)	East of England	Jan-97	22 (22)	NR	No	NR	Yes	Yes	Case control study showed an association between illness and consumption of un-boiled mains water.	Probable
97/035 CDSC (1997)	East of England	Jan-Feb 97	20 (20)	NR	Yes	NR	Yes	Yes	Case control study confirmed an association between illness and consumption of unboiled tap water. Oocysts were detected in untreated water only.	Strong
97/036 CDSC (1997)	East of England	Jan-Feb 97	10 (10)	NR	No	No	-	Yes	-	Possible
97/062 Willocks (1998)	East of England & London	Feb-Apr 97	345 (345)	26	Yes	No	Yes	Yes	Case control study confirmed an association between illness and consumption of mains water derived from a deep chalk borehole supply. The borehole was securely fenced-off, and located next to cattle field and a river 8km downstream of sewage treatment works.	Strong
97/218 CDSC (1997)	North West	May-June 97	346 (346)	NR	No	No	Yes	Yes	Case Control study was inconclusive. Further study limited to Greater Manchester found a significant association between illness and water consumption. The implicated supply combined microstrained & chlorinated surface water and untreated groundwater. Increased turbidity was observed at the aqueduct input following rainfall.	Possible / Strong
Bouchier (2001)	UK	May-97	34	NR	No	NR	NR	NR	This outbreak was possibly linked to a partially filtered spring water supply, potentially contaminated by run off from grazing animals.	Possible
97/493 CDSC (1998)	South East	Nov97-Jan 98	34 (34)	NR	Yes (Smith draft)	No	-	Yes	Inadequate treatment/system failure was identified at the water treatment works.	Strong
98/786 CDSC (1999)	North West	Apr-98	62 (62)	NR	No	Yes	Yes	Yes	Case control study confirmed an association between illness and consumption of mains water.	Strong
Bouchier (2001)	Scotland	Apr-98	303 (303)	NR	Yes	NR	NR	Yes	Outbreak associated with unfiltered mains supplies drawn from Loch Lomond. Dry spell followed by heavy rainfall. Potential contamination from wastewater treatment plant, farm drains and grazing animals.	Probable
99/267 CDSC (1999)	North West	Apr-May 99	347 (347)	NR	Yes	No	-	Yes	The first case occurred six days after a high oocyst count in a 10-litre sample from the water treatment works. Sheep tested positive for *Cryptosporidium*.	Strong
99/577 CDSC (2000)	North East	Sep-Dec 99	28 (28)	4	No	No	-	Yes	The rise in cases was suspected to be related to water but no failure of water quality was detected nor was there any statistical evidence of an association.	Possible

00/219 Howe et al (2002); CDSC (2001)	North West	Mar-00	58 (58)	0	Yes	No	-	Yes	The implicated supply was sourced by spring; well heads had become damaged. Environmental investigation suggested animal faecal contamination. Heavy rainfall and inadequate treatment were potential contributory factors.	Strong
CDSC North West (2000)	North West	Apr-00	207 (207)	NR	No	NR	-	Yes	This outbreak was possibly linke to an unfiltered surface water supply.	Possible
Eastern Health and Social Services Board (2000a)	Northern Ireland	Apr-00	129 (129)	NR	NR	NR	No	Yes	Case control study did not identify any risk factors for illness. Water turbidity increased at the end of April. Several points of potential ingress existed.	Strong
Irvine (2001)	Scotland	May-00	90 (90) 1 death	6	Yes	No	Inconclusive	Yes	Case control study was inconclusive, but the age profile of cases was suggestive of waterborne transmission. Drinking bottled water was protective. The suspected supply was inadequately filtered and showed a slight rise in water turbidity prior to the outbreak.	Strong
Eastern Health and Social Services Board (2000b)	Northern Ireland	Jul-00	168 (168)	NR	Yes	NR	No	Yes	Case control study showed an association between illness and age but not water consumption. Damage to conduit occurred following construction of outflow pipe for a septic tank upstream. Suspected source of the outbreak was ingress of human sewage from septic tank.. Heavy rainfall had occurred.	Strong
CDSC Northern Ireland (2001)	Northern Ireland	Feb-01	306 (306)	NR	Yes	NR	NR	Yes	Oocyst counts were 0-0.62 oocysts/l between February & April. Attack rates were hgher in individuals supplied by the implicated drinking water treatment plant. Several structural failures were identified in the water treatment plant.	Strong
SCIEH (2002a)	Scotland	Jan-02	151 (151)	NR	No	NR	Yes	Yes	Case-control study confirmed association between illness and water consumption. Breaches were detected in 3 sand filters at WTW.	Strong
02/1547 CDSC (2003a)	South East	Nov-02	21 (21)	NR	No	NR	Inconclusive	Yes	Case control study was inconclusive (other sources identified as risk factors). No WTW problems identified. Cases received water from two companies, solely or in combination. Continuous monitoring has occasionally detected low levels of oocysts in water from these companies.	Possible
02/1701 CDSC (2003b)	South East	Nov-Dec 02	31 (31)	NR	Yes	No	-	Yes	Heavy rain in water catchment area occurred prior to the outbreak. Treated water supply was fed by river and borehole supplies.	Strong
05/552	South East	Sep-Nov 05	140 (140)	5	Yes	No	On-going	Yes	Case control study on-going. Increase in incidence among consumers supplied by one water source (surface), but not other 3 (groundwater). Implicated supply draws water from river into which treated sewage is discharged. Low water levels may have reduced dilution. Case-control study on-going. Failure in water treatment system.	Strong
CDSC (2005b)	Wales	Sep-Nov 05	100 (100)	NR	Yes	NR	Yes	Yes	Case control evidence showed an association between illness and water consumption from implicated surface water supply. *C.* hominis oocysts were detected in sewage effluent, raw water and treated water in distribution. Sewage effluent in catchment area is hypothesized as the outbreak source.	Strong

Appendix 15. UK outbreaks of *Cryptosporidium* involving private water supplies in the UK: 1983-2005

Reference/ CDSC Code	Region	Date	No. of cases in outbreak	Hospit-al cases	Pathogen in Water	Faecal Indicator Organisms in water	Analytical Epidemiology	Descriptive Epidemiology Association	Additional Information	Strength of Association
92/149	North West	Apr-May 92	42 (12)	0	No	No	-	Yes	Outbreak possibly linked to a school's private water supply Slurry spraying occurred near the reservoir.	Possible
93/175	Wales	Apr-93	9 (1)	1	No	No	-	Yes	Cases drank well and spring water while camping and also had contact with animals.	Possible
93/168 Duke et al (1996)	North East	May-93	43 (7)	2	No	Yes	-	Yes	Campylobacter and suspected *Cryptosporidium*. Outbreak at a university hall of residence with an un-chlorinated surface water supply. UV and filtration in place but accumulation of iron deposits obscured lamp covering. Dead lambs found in water supply system.	Probable
98/268 CDSC (1999)	North West	Mar-Apr 98	24 (6)	1	Yes	No	-	Yes	Outbreak was associated with drinking water from a private tank supplied by mains water. The water tank was in poor condition and contaminated with sheep droppings.	Strong
00/440 CDSC (2001)	South West	May-June 00	8 (3)	NR	No	Yes	-	No	Outbreak may have been linked to an untreated/ partially treated water supply at a farm holiday centre. Also possibly linked to a recreational stream.	Possible
02/018 CDSC (2002)	Yorkshire and Humber	Mar-02	50 (1 Cryptos-poridium)	NR	No	No	-	Yes	Mixed outbreak of Campylobacter and *Cryptosporidium*. Previous outbreaks of campylobacter and *Cryptosporidium* were linked to the same private well in 1992 and 1993.	Possible

Appendix 16. Forty seven outbreaks of *Cryptosporidium* involving UK recreational water: 1983-2005

Reference/ CDSC code	Suspected Source of outbreak	Region	Date	No. of cases in outbreak (lab positive)	Hospital Cases	Pathogen in Water	Faecal Indicator Organisms in water	Analytical Epidemiology Association	Descriptive Epidemiology Association	Additional Information	Strength of Association
Joce et al (1991)	Swimming pool	Yorkshire and Humber	Jun-Oct 88	79 (79)	NR	Yes	NR	Yes	Yes	Problems controlling chlorine levels occurred prior to outbreak. Sewage contaminated learner pool through broken drainage connection with public toilets at pool. Case-control study showed association between illness and head immersion in swimming pool. Secondary person-to-person transmission occurred.	Strong
Hunt et al (1994) 92/079	Public swimming pool	South West	Mar-92	13 (13)	2	Yes	Not tested	No	Yes	Descriptive epidemiology indicated nine cases were associated with visiting a swimming pool, in which a faecal incident had occurred.	Strong
93/067	School swimming pool	West Midlands	Jan-Feb 93	23 (21)	4	No	No	No	Yes	Outbreak was linked to a school swimming pool.	Possible
94/453	Private swimming pool	East of England	1994	3 (NR)	NR	Yes	No	No	No	Outbreak linked to a country club swimming pool. No records of pH, maintenance or faecal incidents.	Probable
94/454	Public swimming pool	East of England	1994	4 (NR)	NR	Yes	No	No	No	-	Probable
94/347 CDSC (1995)	Public swimming pool	South West	Oct-Nov 94	14 (8)	0	No	No	No	Yes	Descriptive epidemiology suggested the outbreak was linked to a public swimming pool.	Possible
95/754	Paddling pool in swimming complex	East Midlands	Aug-95	3 (3)	3	No	No	No	Yes	Outbreak was possibly linked to a paddling pool at a public leisure centre. Cleaning and supervision of the pool were inadequate.	Possible

96/569 Sundkvist et al (1997)	Swimming Pool	South East	Jul-Aug 96	8 (8)	NR	No	No	Yes	Yes	Case-control study suggested an association between the frequency of total immersion in pool and illness. No treatment problems were idenfified. *Enterobius* ova detected in filter backwash indicates faecal contamination.	Probable
97/203	River exposure	North West	May-97	13 (7)	0	No	No	No	Yes	Outbreak possibly linked to river exposure at a residential activity centre. Water ran off adjacent fields into river during heavy rainfall.	Possible
97/309	Public swimming pool	South East	May-97	9 (9)	NR	No	No	No	Yes	Learning pool at public leisure centre was implicated in this outbreak. Ozone generator was faulty therefore pool relied solely on chlorine disinfection.	Probable
98/181 CDSC (1999)	Private swimming pool	London	Mar-98	6 (6)	NR	No	Yes	No	Yes	Outbreak was linked to swimming pool at private members' club. Chlorination and filtration were defective. Water from children's pool contaminated the adjacent adult pool.	Probable
98/631 CDSC (1999)	Swimming pool	East of England	Sep-Nov 98	9 (9)	NR	No	Yes	No	Yes	Symptomatic individuals used the pool and ozone treatment failed.	Probable
98/626 CDSC (1999)	Swimming pool	South East	Sep-Nov 98	14 (11)	3	No	NR	No	Yes	Descriptive epidemiology suggested outbreak was linked to a public swimming pool complex.	Possible
99/255	Public swimming pool	South West	Jul-99	11 (11)	0	No	No	Yes	No	Case-control study indicated association between illness and visiting a swimming pool. It was suspected that an infected individual used the pool. No filtration problems occurred at the time of the outbreak.	Probable
99/582	Public swimming pool	South East	Aug-Nov 99	54 (NR)	1	Yes	No	Yes	Yes	Case-control study confirmed association between illness and using swimming pool.	Strong
99/583	Private swimming pool	West Midlands	Aug-Oct 99	16 (14)	1	Yes	No	No	Yes	High level of contamination were detected (188 oocysts/10L).	Strong

99/600	Public swimming pool	East Midlands	1999	16 (16)	NR	No	No	Yes	No	Probable faecal accident occurred. Detected *Giardia* in water samples but no *Cryptosporidium* oocysts.	Probable/ Strong
99/669	Swimming pool	South East	Nov-99	4 (NR)	0	No	Yes	No	No	Defecation incident occurred in the pool. Junior pool was emptied and cleaned. Main pool drained routinely. Increased sampling of filter backwash. Advised more stringent cleaning after faecal incidents.	Possible
99/741	Swimming pool	London	1999	30 (11)	NR	No	No	Yes (no details)	No	Following the outbreak the pool was closed for refurbishment. Sand filters were emptied and refilled with new media.	Probable
CDSC 99/679	Public swimming pool	West Midlands	Sep-99	8 (8)	NR	No	No	No	No	Environmental contamination was reported to have occurred prior to the outbreak.	Possible
00/1022 CDSC (2001)	Public swimming pool	East of England	Sep-00	7 (7)	NR	No	No	No	Yes	Two viable oocysts were isolated from a sand filter (surface sand from teaching pool). Improved operational procedures were introduced.	Possible
00/1023	Public swimming pool	East Midlands	Sep-Oct 00	9 (9)	0	No	Yes	No	No	Possible increased bacterial load in the teaching pool. Filters were backwashed, & chlorine level increased. Water in main pool was resampled.	Possible
00/406	Public swimming pool	Yorkshire and Humber	May-Jun 00	41 (41)	2	Yes	No	No	Yes	Increase in bacterial load challenged the system. Pool was subsequently closed, water filtered and backwashed.	Strong
CDSC 00/656 CDSC (2001)	Private swimming pool	London	Sep-00	10 (10)	0	Yes	Yes	No	No	Faecal incidents and poor pool management. One of 2 pools was closed. Fitlers were cleaned and backwashed. Public information produced.	Probable
00/723 CDSC (2001)	Public swimming pool	London	Jul-Aug 00	5 (5)	NR	Yes	NR	NR	NR	Oocysts were detected in pool water and filter. Pool operations were reviewed and improved.	Probable
00/870	Swimming pool	South West	Sep-Oct 00	12 (7)	0	Yes	No	No	Yes	Cases used pool.Small pool may have leaked/taken in untreated water. System was flushed and water/sand was tested for oocysts pre and post backwashing.	Strong
00/972 CDSC (2001)	Private swimming pool	South West	Oct-Nov 00	5 (5)	NR	No	NR	No	Yes	Heavy rain occurred prior to the outbreak. One viable oocyst was detected in a filter sample. Pool was chlorinated, closed and drained. Filters backwashed. Resampling gave negative results.	Possible

01/136 CDSC (2002)	Public swimming pool	South East	Feb-Mar 01	5 (4)	0	Yes	No	No	Yes	Inadequate treatment of water. Oocysts were detected in learner and main pools. Control: coagulation and filtration employed. Filters backwashed. Pool and surrounding area cleaned.	Strong
01/347	School outdoor swimming pool	South East	May-01	152* (10)	1	Yes	No	Yes (no details)	Yes	*Proportion of symptomatic cases may have been due to concurrent community norovirus outbreak. System chlorinated. Pool temporarily closed.	Strong
01/440	Recreation-al water	South West	Aug-01	14 (5)	NR	No	Yes	No	No	Outbreak linked to recreational water use at a beach. Water treatment plant fed into stream. Possible illegal tipping into sewer from chemical toilets on campsite.	Possible
01/528	Public swimming pool	South West	Oct-Nov 01	3 (3)	NR	Not tested	No	No	Yes	Advice given on improved pool operation and management.	Possible
02/1877	Public swimming pool	South East	Sep 02 – Feb 03	20 (20)	NR	No	Yes	No	Yes	Oocysts detected in pool sand filters. Increased bacterial load suggested faecal accident. Person-to-person transmission also occurred. Control measures: increased chlorination of pool, hyperchlorination of filters and balance tank.	Probable
02/302 CDSC (2003b)	Swimming pool	North West	Apr-02	4 (3)	0	No	Yes	No	Yes	Mixed outbreak of *Cryptosporidium* and Rotavirus associated with swimming pool at a holiday camp. Probably also linked to borehole drinking water supply.	Probable
SCIEH (2002c)	Swimming pool	Scotland	Aug-02	12 (8)	NR	NR	NR	NR	Yes	Faecal accident had occurred in the pool.	Probable
HPS Scotland (2002b)	Swimming pool complex	Scotland	Oct-Nov 02	7 (7)	NR	NR	NR	NR	Yes	Descriptive epidemiology suggestive of association between illness and swimming at leisure centre.	Possible
03/220	Public swimming pool	Yorkshire and Humber	Jan-03	66 (48)	0	Yes	No	No	Yes	Suspected faecal contamination of pool. Output from training pool filter was 8 oocysts/10l. Flocculation was intensified and filters backwashed as control measures.	Strong
03/400	Public swimming pool	South West	Aug-Oct 03	22 (1)	0	No	No	No	Yes	Outbreak of *Cryptosporidium* and *Giardia* associated with public swimming pool.	Possible
03/401 CDSC (2004)	Interactive water feature	South West	Aug-03	63 (31)	10	No	Yes	Yes	Yes	Outbreak associated with 'water splash zone'. Water heavily contaminated with faecal coliforms. Re-circulation of water with inadequate filtration/ disinfection. Attraction was closed.	Probable

SCIEH (2003a & b)	Swimming pool	Scotland	Aug-03	28 (17)	NR	Yes	NR	NR	Yes	Descriptive epidemiology evidence suggested an association between illness and visiting a public swimming pool. Oocysts detected in pool water. Pool heavily used at this time of year.	Strong
03/409	Public swimming pool	South West	Jul-Sep 03	17 (17)	1	Yes	No	No	Yes	Oocysts detected in learner pool. Control measures: pool hyper-chlorinated and filters backwashed.	Strong
03/410	Public water feature	South West	Jul-Aug 03	4 (4)	0	Yes	No	No	Yes	Outbreak associated with an untreated water fountain. Control measures included refurbishments, improved management and increased display of information signs.	Strong
03/411	Public water feature	West Midlands	Jul-Sep 03	122 (35)	2	No	Yes	No	Yes	Outbreak associated with water fountain in public park. Treatment system failed under increased bacterial load. The water feature was subsequently closed.	Probable
03/546	Private swimming pool	South West	Oct-03	2 (2)	0	No	Yes	No	Yes	Swimming pool system was flushed following detection of the outbreak.	Probable
04/281 CDSC (2006)	Public swimming pool/ person-to-person	East	Mar-Apr 04	13 (9)	0	Yes	No	No	Yes	Index case suspected to have become infected on farm visit. This case attended nursery and nursery children then visited swimming pool. As a control measure sand filters were changed.	Strong
04/186 CDSC (2006)	Public swimming pool	Yorkshire & Humber	May-Jun 04	7 (7)	0	No	No	No	Yes	Control measures: filters backwashed, pool management improved according to operators' guidance and HPA advice.	Possible
04/446 CDSC (2006)	School swimming pool	West Midlands	Oct-Nov 04	12 (9)	0	Yes	No	No	Yes	Outbreak associated with school swimming pool used by community groups. Pool closed, emptied, disinfected. After backwashing, filters tested negative for oocysts.	Strong
04/371 CDSC (2006)	Public swimming pool	Yorkshire & Humber	Oct-Nov 04	10 (9)	0	Yes	No	No	Yes	Outbreak associated with leisure centre swimming pool. Control measures taken: pool water cycled and sand filters backwashed. External inspection showed large cartridge filter samples negative for oocysts.	Strong

04/659 CDSC (2006)	Public swimming pool	West Midlands	Oct-Nov 04	6 (6)	0	No	Yes	No	Yes	Filter inspection window had become dirty and discoloured and was replaced. Introduced system for recording pool incidents and contamination.	Probable
05/410	Public swimming pool	South West	May-05	3 (2)	0	No	No	No	Yes	Control measures: system chlorinated and frequency of filter backwashing increased.	Possible
05/514	Children's paddling pool at animal park	East	Jul-05	10 (3)	0	No	Yes	No	Yes	Pool water showed increased bacterial load and turbidity. Filters changed as a control measure.	Probable

Appendix 17. Other outbreaks of *Cryptosporidium* in the UK: 1983-2005

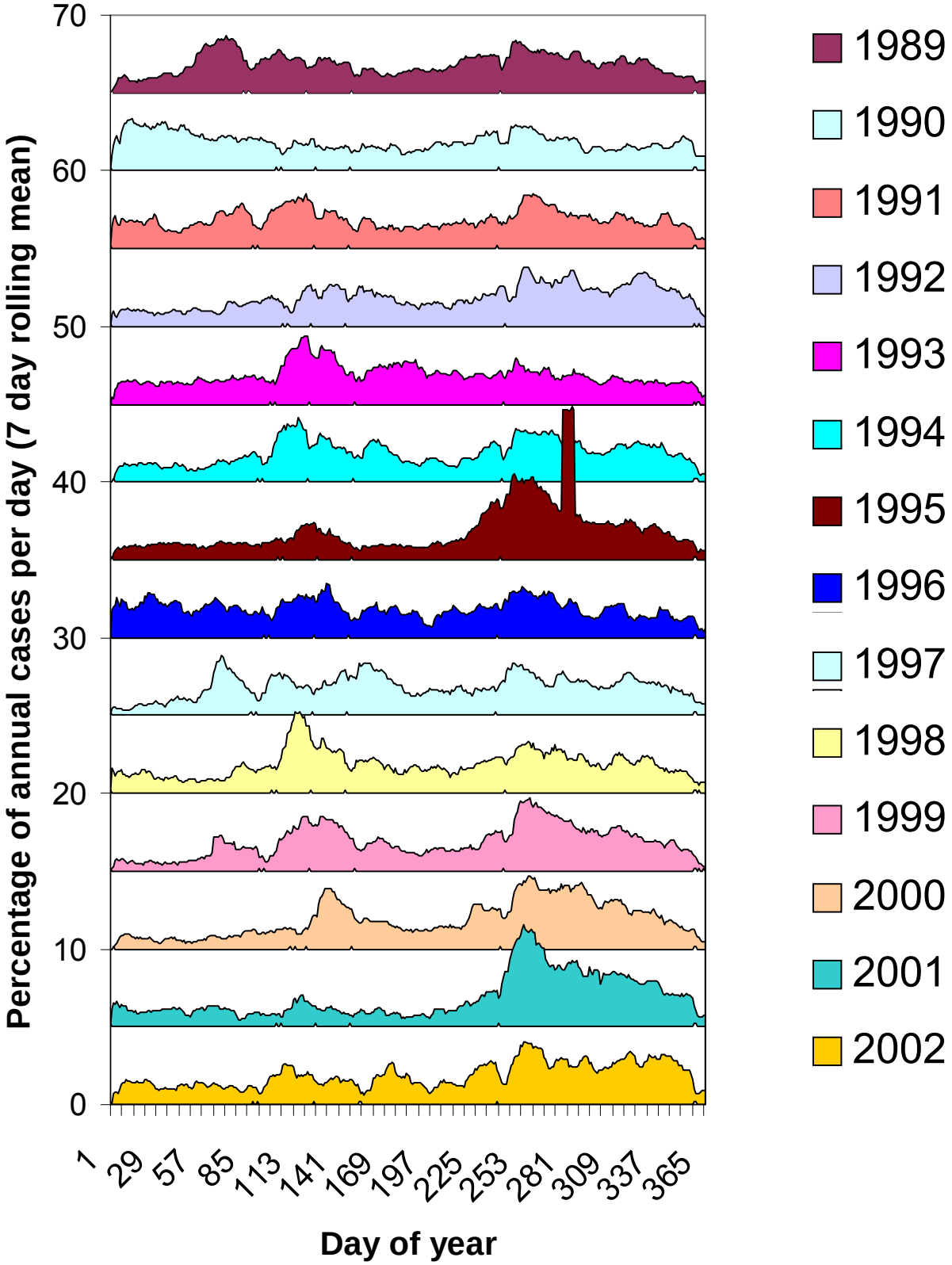
Code/ Reference	Suspected mode of transmission	Region	Date	No. of cases in outbreak (lab positive)	Hospital Cases	Pathogen detected in implicated vehicle	Analytical Epidemiology Association	Descriptive Epidemiology Association	Additional Information
Brown et al (1989)	Person-to-person	East of England	Apr-May 86	36	NR	No	Case-control study provided no evidence of waterborne transmission	Descriptive epidemiology suggested no common source of infection	Slurry spraying on frozen ground in early spring. Followed by heavy rainfall.
92/125	Animal contact	East Midlands	Apr-92	10 (2)	0	No	-	-	Outbreak associated with contact with farm animals.
92/124	Unknown	North West	Apr-Jun 92	33 (33)	0	N/A	-	-	-
93/097	Animal contact	South East	Apr-93	60 (60)	8	N/A	-	-	Lambs suspected as the source of infection.
93/125	Animal contact	South East	Apr-May 93	100 (66)	9	Yes	Case-control study confirmed association between outbreak and contact with lambs	-	-
93/128	Unknown	North West	Apr-May 93	16 (15)	1	N/A	-	-	-
94/125	Animal contact	Yorkshire and Humber	Mar-Apr 94	541 (40)	NR	NR	Cohort study confirmed association between illness and contact with farm animals.	Yes	Outbreak associated with a farm in Lincolnshire.
94/024	Food borne followed by person-to-person	North West	Jan-94	13 (13)	0	N/A	-	-	No food vehicle identified.
94/178	Food borne followed by person-to-person	North West	Jan-Jun 94	50 (50)	NR	N/A	-	-	No food vehicle identified.

Evans et al (1997) 95/411	Animal contact	Wales	Apr-95	33 (7)	1	No	Cohort study confirmed association. Risk factors for illness: contact with calves and habitually sucking thumbs or biting nails.	-	Several calves diarrhoeic. Pens and hand rails heavily contaminated with faeces. Inadequate washing facilities available.
Djuretic et al (1997) 95/781	Food-borne	Yorkshire and Humber	Sep-95	67 (16)	2	No	Case-control study showed association between consumption of milk and illness	-	Outbreak associated with consumption of milk at a junior school supplied by a farm. Inadequate pasteurisation (failed phosphatase test).
95/478	Linked to farm	North East	Apr-Mar 95	21 (15)	2	N/A	-	-	Outbreak linked to a farm.
95/787	Unknown	South East	Sep-Oct 95	12 (12)	NR	N/A	-	-	Community outbreak.
96/145	Unknown	North West	Feb-96	17 (11)	NR	N/A	-	-	Community outbreak.
97/079	Animal contact	North West	Feb-Mar 97	20 (7)	3	No	-	-	Outbreak linked to an educational farm visit during which students fed newborn lambs. Inadequate washing facilities available.
97/298	Animal contact	North Wales	Apr-97	9 (9)	2	No	-	-	Outbreak linked to a school farm trip, during which students handled animals.
97/428	Person-to-person	South West	Oct-Nov 97	31 (30)	0	N/A	-	-	Outbreak linked to a nursery. Children bathed communally. Inadequate precautions were taken after handling infected individuals.
97/215	Unknown	South West	May-Aug 97	70 (70)	NR	N/A	-	-	Outbreak occurred in multiple areas in the South West region.
98/157	Person-to-person	Yorkshire and Humber	Jan-Feb 98	12 (10)	NR	N/A	-	-	Mixed outbreak of Rotavirus and *Cryptosporidium*, linked to a nursery.
99/156	Animal contact	North Wales	Mar-99	30 (8)	NR	No	-	-	Outbreak suspected to be linked to animal contact on farm. Lack of hand washing after handling animals.
99/180	Person-to-person	South West	Mar-Apr 99	11 (11)	NR	N/A	-	-	Outbreak occurred in a school. Person-to-person spread suspected.
00/1040	Animal contact	North West	Nov-00	4 (2)	0	No	-	-	Outbreak suspected to be linked to a dairy farm.

00/806	Person-to-person	London	Oct-00	13 (13)	1	N/A	-	-	Outbreak linked to a nursery. Poor personal hygiene reported.
01/442	Person-to-person	South East	Aug-Sep 01	30 (10)	0	N/A	-	-	Outbreak linked to a nursery.
02/1794	Person-to-person	Yorkshire and Humber	Nov-Dec 02	47 (12)	NR	N/A	-	-	Outbreak linked to a nursery.
03/167	Animal contact	East of England	Feb-03	6 (6)	0	No	-	-	Outbreak suspected to be linked to contact with calves and scouring lambs on a farm.
03/197	Animal contact	North Wales	Mar-03	17 (6)	0	No	-	-	-
03/252	Food borne	West Midlands	Feb-03	10 (2)	0	No	-	-	Outbreak linked to an adult training centre. No food item implicated.
03/315	Person-to-person	Yorkshire and Humber	May-03	68 (22)	0	N/A	-	-	Outbreak linked to a nursery.
03/438	Person-to-person	South West	Sep-Oct 03	20 (4)	0	N/A	-	-	Outbreak linked to a nursery.
04/089	Animal contact	Wales	Mar-04	20 (6)	NR	N/A	-	-	Outbreak associated with exposure to farm animals (possible link to infected goat).
04/241	Animal contact	South West	Mar-04	20 (9)	2	Yes	-	Yes	Outbreak associated with exposure to calves on a farm.
04/484	Animal contact	Wales	Nov-04	2 (2)	0	N/A	-	-	Infected individuals had spent five days on a farm, cleaning and feeding animals.
04/557	Person-to-person	East	Nov-04	11 (2)	0	N/A	-	-	Outbreak linked to a special needs school. Poor facilities reported.
05/208	Animal contact	Wales	Jan-05	2 (2)	1	N/A	-	-	Outbreak linked to contact with animals during a farm visit.
05/409	Linked to farm	South West	May-05	2 (2)	0	N/A	-	-	Outbreak linked to camping on a farm. Sheep present on the camping field. No shower facilities available.
05/076	Linked to farm	South West	Jan-05	2 (2)	NR	N/A	-	-	Outbreak occurred on a farm.

HPS Scotland (2005)	Animal contact	Scotland	Apr-05	62 (62)	6	NR	-	Yes	Outbreak linked to wildlife centre and suspected to be associated with animal petting. Advice given on hygienic practices and hand-washing.

Appendix 18. The seasonality of Cryptosporidiosis in England and Wales



The anomaly seen in 1995 represents a large number of cases from the Torbay outbreak that were reported on the same date but without a specimen date. Bank Holidays are shown as nicks in the x axis.

Appendix 19. *Cryptosporidium* cases per day 1989-2006 by the date of the faecal sample