Executive Summary

Background

Cryptosporidium parvum, a protozoan parasite, is an important cause of gastro intestinal illness in normal, as well as immunocompromised individuals. It is now the third most common, non-viral cause of gastroenteritis in England and Wales found in about 2% (range 0.5 to 3.9%) of all faecal specimens examined in laboratories. Between 1991 and 2000, laboratories in England and Wales reported an average of 4761 cases annually. Certain regions and districts report consistently high incidences of cryptosporidiosis, which are not attributable to variations in laboratory protocols. In some years the high incidences may be explained as part of documented outbreaks, these being the subject of rigorous investigations. However, detailed analytical studies have not been conducted into routine sporadic cases to ascertain the sources of the infection.

Introduction

In 1992, an outbreak of cryptosporidiosis occurred in Allerdale and Copeland, two local authorities in North Cumbria. A case-control study conducted during the outbreak showed a statistically significant association between cases of cryptosporidiosis and consumption of mains water from Ennerdale. Prior to 1 March 2000, Ennerdale and Crummock water zones supplied unfiltered water while the rest of the water supply zones in the two local authorities provided a variety of conventionally treated (coagulation and sand filtration) and unfiltered water. Following the outbreak, high levels of sporadic cases persisted in Allerdale and Copeland when compared with the neighbouring local authorities of Carlisle City and Eden.

A prospective case-control study was initiated on 1 March 1996 to examine risk factors in primary sporadic cases of cryptosporidiosis within Allerdale and Copeland and also set out to test the hypothesis that there is a dose-response relationship between the risk of gastroenteritis due to *Cryptosporidium* and the consumption of mains water in the Crummock and Ennerdale water supply zones. The case-control study was initially scheduled to cover a period of two and half years.

The project was then extended for a further four years to incorporate an Intervention Study, when it transpired that membrane filters were to be commissioned at the Ennerdale and Cornhow treatment works. (The Cornhow treatment works being the treatment works for the Crummock water supply zone). This latter study examined whether there were differences before and after the installation of membrane filters, in terms of the cryptosporidiosis incidence rates, risk factors, and the dose-response relationship between cases of cryptosporidiosis and consumption of mains water in the Ennerdale and Crummock water supply zones. The membrane filters were subsequently installed between 1 March and 31 July 2000. The period before the installation of membrane filters, 1 March 1996 to 29 February 2000 was the pre-intervention phase and was also the designated period for the initial case-control study. The post intervention period extended from 1 August 2000 to 31 August 2002, when data collection ended.

Method
Epidemiological Investigations

The same method was consistently applied throughout the six and half years. Cases were defined as residents of Copeland and Allerdale, who had diarrhoea, and microbiologically confirmed cryptosporidiosis during the period of study. They were primary cases who had not travelled abroad in the two weeks before illness. Secondary cases within the same household were excluded. Controls were residents in the same local authority area, with no diarrhoea and who had not travelled abroad in the two weeks prior to interview. They were identified at random from the GP register, loosely comparable to the case in three broad age bands 0-5 years, 6-15 years, 16+ years and similar post code sector. The study was designed for three controls per case. The case control and intervention studies covered a period of six and a half years, 1 March 1996 to 31 August 2002. Both East and West Cumbria Health District Ethics of Research Committees approved all parts of the research.

A research assistant and deputies followed a set protocol for contacting and interviewing the cases and controls. Standard letters with appointment times were sent, followed by face to face interviews at home. A specially designed questionnaire was used which incorporated personal details, travel, exposures to other cases, consumption of dairy products, salads, raw meats and sausages, pasteurised and unpasteurised milk, water, participation of recreational activity, contact with farm, farm animals, slurry, day care facilities and type of sewerage. A picture of a cup, glass, tumbler, mug was shown to interviewees to assess liquid consumption. Following the interview, an information leaflet on cryptosporidiosis and the research was given to interviewees; the hypothesis on dose response was not mentioned in all the information to the public. Interview times were routinely recorded. Completed questionnaires were sent to the PHLS Statistics Unit for data analyses. Cases and controls that had previously been interviewed were not reused; thus avoiding duplication and data bias.

The study was carried out in two local Authority areas: Allerdale and Copeland. Postcode location of cases and controls were transposed onto the different water supply zones and to be co-terminus with: Ennerdale, Crummock and "Other" (defined as supply areas outwith these two). This was done for both studies and essential in the intervention study, in which the comparison of the effects of the membrane filters was to be strictly applied to the Ennerdale and Crummock water supply zones. In addition, mixed water supply areas could also be identified; this allowed data collected regarding Ennerdale, and Crummock water supply zones to be analysed exclusively.

Data Analysis
Case-Control Study
(1 March 1996 to 29 February 2000)

Single variable analysis was first conducted on each potential risk factor to estimate odds ratio and 95% confidence intervals (The risk factor is the risk among those exposed to the risk factor; the odds ratio is the odds of the disease to non disease in those with the risk factor). Dose response was investigated using chi-square test for trends. Analyses were conducted using the whole data set and then excluding individuals receiving mixed water supply.

Variables with positive association with illness reaching 20% significance level were included in a logistic regression model. Terms were assessed by comparison of nested models

using likelihood ratio tests. Variables were then removed from models if their corresponding p value was 0.3 or greater, but the variables of age, sex and water supply zones were retained, irrespective of their statistical significance. Analyses were conducted using the statistical software packages Epi-info and GLIM.

Intervention Study

Incidence rate calculations

Primary cases fulfiling the study criteria were allocated to the relevant water supply zones: Ennerdale, Crummock and "Other" the water supply zones outwith Ennerdale and Crummock but within the local authority boundaries. Ennerdale and Crummock water had new Membrane Filters installed in the latter part of the study period; the "Other" water supply zones did not have any major changes to water treatment during the whole study period. Cases were then subdivided into five time frames.

(1) Pre-installation of membrane filters (1 March 1996 to 29 February 2000).
(2) Commissioning of membrane filters (1 March 2000 to 31 July 2000).
(3) Established membrane filtration prior to Foot and Mouth (FMD) epidemic (1 August 2000 to 20 February 2001).
(4) Established membrane filtration and FMD epidemic (21 February 2001 to 20 January 2002).
(5) Established membrane filtration following FMD epidemic (21 January 2002 to 31 August 2002).

Denominator populations of water supply zones were estimated from information supplied and agreed by the water company. Estimation of incidence rates per 100,000 person years and 95% confidence intervals were calculated for the five different time intervals as listed above, for the defined water supply zones. Incidence rate comparisons were then made of those recruited in the period before and after the installation of membrane filters, for the relevant water supply zones.

A Poisson Regression model was constructed with the number of cases of cryptosporidiosis as the predictor variable and the number of person years of observation on the offset. The independent effect of the Membrane Filtration installation and for the time period before and after Membrane filtration installation were determined separately. The interaction between the two was used. Two models were applied; one which included the commissioning period, the other without it.

Risk Factors Analysis

Changes in risk factors for sporadic cryptosporidiosis during the five time periods of observations as listed previously were undertaken. Initially, exploratory analysis was conducted. Five sets of contingency tables were constructed, one for each time period of observation, for each exposure and odds ratios calculated. Variables significant at $p < 0.2$ in any of the five sets of contingency tables were returned for inclusion in logistic regression models. A sequence of multivariable models was fitted removing clearly non-significant variables. An interaction term between the five time intervals of the study and each of the remaining variables was included in separate multivariable models to identify a subset of interactions for evaluation together in further models. Non-significant interactions were removed in order of least significance. The final model consisted of a single interaction term of total daily volume of tap water consumed per day in each of the five intervals of the study, main effects variables, age, sex and water zone.

Microbiological Investigations

Analysis of stool specimens was carried out by three local laboratories, which included the local Public Health Laboratory. Isolates were sent to the *Cryptosporidium* Reference Unit for confirmation and genotyping.

Results
Case control study
- 1 March 1996 to 29 February 2000
Reported cases

In the four years of the sporadic case-control study (1996-1999), the number of all laboratory reported cases of cryptosporidiosis in the study area; Allerdale and Copeland, averaged 52 cases per year (rate = 31.2 per 100,000). The highest number, 89 cases (rate=53.9 per 100,000) was seen in 1999 and the lowest number 24 cases (rate = 14.4 per 100,000) was seen in 1997. Allerdale and Copeland consistently showed high incidence rates. The lowest rate in 1997 in the study area was still about one and a half times higher than the regional and national rates.

Between 1 March 1996 and 29 February 2000, two hundred and seven laboratory confirmed cases of cryptosporidiosis were reported to the research assistant. Of the 207 cases, 55 (26.6%) were not in the study because they did not fulfil the study criteria, refused to participate or could not be found. Two notable categories, which did not fulfil study criteria, were travel abroad cases 6(2.9%) and secondary cases 36(17.4%). One case refused to participate, one could not be interviewed and two who did not respond after repeated efforts to contact them; these four cases could be combined into the category of "non-responders" (2%). Similar exclusion criteria were applied to controls.

Recruited Primary cases

Thus, a total of 152 primary cases and 466 controls were entered into the study, of these 82 (54%) cases and 236 (51%) controls were male. Three hundred and thirty two (59%) of the cases and controls were aged five or under and only 64 (11%) were over 16 years. The mean and median ages of all cases were eight and four years respectively and ranged between <1 to 55 years of age. The main symptoms were diarrhoea, abdominal pain and vomiting with the median duration of illness lasting 9 days. Twenty- three (15%) of the cases were admitted to Hospital; all these were children (median age = 2 years; range <1 to 14yrs).

Single and multivariable analyses were carried out in different models, first on total water consumed per day; using all data, and excluding mixed water supply. An additional multivariable model examined home tap water consumed (all data). All the models showed a highly significant association between cases of cryptosporidiosis and drinking unboiled tap water. The odds per pint of becoming infected with *Cryptosporidium* from drinking total unboiled tap water was approximately 1.40; the 95% confidence interval being 1.14 to 1.71 (p for trend =0.001).

The dose response relationship for total water consumed showed that the odds of contracting cryptosporidiosis increased by 40% with each extra pint of unboiled water consumed daily. There was no evidence of a significant difference in the risk of becoming infected from consuming unboiled tap water in the different water zones; irrespective of whether the supply was unfiltered, varieties of conventionally filtered (coagulation and sand filtration) or private. The results were virtually

the same if consumption of unboiled home tap water was used instead of total consumption of unboiled tap water.

A significant association was found with *Cryptosporidium* infection and short farm visits, contact with farms, farm animals, animal faeces, any other contact with farm animals, stroking animals on the single variable analysis. Multivariable analyses showed short farm visits (p = 0.04) and frequency of farm contact (p for trend =0.02) to be significant.

There was a significant association between cases and eating food within 1 to 2 hours after contact with farm animals, in the single variable analysis. After contact with farm animals, washing hands before eating appears to protect against the infection. A protective effect was also seen with the consumption of pasteurised milk (odds ratio 0.55 (0.33, 0.91) p=0.02).

All other variables examined were not found to be risk factors for sporadic cryptosporidiosis including salads, dairy products, raw sausages, raw meats, pasteurised or unpasteurised milk, ice, bottled water, soft drinks, contact or walk past slurry, swimming, water sports or paddling, contact with playgroup, contact with anyone with diarrhoea (outside household), travel in UK, or having different sewage systems.

Intervention Study
- 1 March 1996 to 31 August 2002
Reported cases

Of the total number (249) of laboratory confirmed cases reported to the RA for the six and a half years period, 207 occurred in the pre intervention period (1 March 1996 - 29 February 2000), 11 cases in the membrane filter commissioning period (1 March 2000 - 31 July 2000) and 31 in the post intervention period (1 August 2000 to 31 August 2002).

After accounting for secondary cases, travel abroad, and those who refused to participate, there were 153 primary cases in the pre intervention period, 4 in the commissioning period, and 18 in the post intervention period. The marked decrease in cases was seen in year 2000 and continued into year 2001 and 2002 in the post intervention period. The reduction was proportionately greater in primary than secondary cases. The Foot and Mouth epidemic had a marked effect in the study area in 2001.

Recruited Primary cases

A total of 175 primary cases and 537 controls were recruited for the whole six and a half year period. Of the 175 primary cases, 90 were male and 85 female. One hundred and fifty three cases occurred in the pre intervention period (1 March 1996-29 February 2000); the locations of these primary cases showed 56 in Crummock water supply zone, 43 in Ennerdale water supply zone, and 54 in "Other". In the post intervention period (1 March 2000 to 31 August 2002), there were six cases in Crummock, three in Ennerdale and 13 in "Other" water supply zones.

The annual incidence rate of primary sporadic cryptosporidiosis exceeded 10 per 100,000 in the pre intervention period from 1996 to 2000 with a peak of 35 per 100,000 in 1999. In the post intervention period, the incidence declined to below 10 per 100,000 in year 2000, 2001, and 2002 to the end of recruitment in August 2002.

The majority of cases in the total period were under 16 years: 88% in the pre intervention period and 73% in the post intervention period. Comparison of age groups of cases showed 57% cases in the pre intervention period and 41% in the post intervention period to be children under five years.

The mean and median age of the total number of cases (175) was 9.1 and 4 years respectively (range <1 to 62). In the pre intervention period, the mean and median age of cases were 8 and 4 years (range <1 to 55) respectively; in the post intervention period, these were 16.6 and 8 years (range 1 to 62).

Diarrhoea and abdominal pain were the two most common symptoms among cases in both periods. The median duration of symptoms of illness was 9 days (range 2 to 21) in the pre intervention period and 10 days (range 3 to 20) in the post intervention period respectively.

In the pre intervention period 23 (15%) cases were admitted to hospital, and their ages were <1 to 14 years with mean and median age of 3.7 and 2 years respectively. In the post intervention period, three cases (14%) were admitted to hospital and their ages ranged from 3 to 12 years with the mean and median age of 8.3 and 10 years respectively.

A seasonal spring peak was apparent in cases in the pre intervention period between 1996 and 2000, but was no longer conspicuous following the introduction of Membrane Filtration in Ennerdale & Crummock.

The incidence rates per 100,000 person years in the populations served by Crummock, Ennerdale, and "Other" water sources were similar during the pre intervention period. There was a considerable reduction in incidence rates of sporadic cryptosporidiosis during and after commissioning of Membrane filtration; as well as during and after the FMD epidemic, which was apparent for each of the water sources, reflecting both installation of Membrane Filtration and the impact of the FMD epidemic in livestock. The third of the population living in the "Other" supplies zones continued to receive water from a variety of conventionally treated and unfiltered sources. The decline in this "Other" group zone, may therefore be attributed to the FMD epidemic alone. Ennerdale and Crummock water supply zones experienced a decline in incidence rate during and following installation of filters; this decline was in excess of that which could be explained by the FMD epidemic and may therefore be attributed to the installation of Membrane Filtration. Poisson regression was used to examine this aspect.

Poisson Regression

Two models were applied; one included the commissioning period of the Membrane Filters, the other omitting this time period.

In the first model which included the commissioning (1 March 2000 to 31 July 2000) and the post commissioning period (1 August 2000 to 31 August 2002), the results showed a significant interaction between the two predictors (p = 0.024) indicating that the decrease in incidence in the water zones with membrane filtration was significantly different from the decrease in incidence in the "other" water zones. In the second model, the commissioning period was ignored. The results showed a slightly diminished interaction (p = 0.07).

The estimated incidence rate ratio for the "Other" zones (with no changes throughout the study period) was 0.387 (95% C1 0.197

to 0.759) for the time period before and after membrane filtration in Crummock and Ennerdale. This could be interpreted as the independent effect of the FMD epidemic.

For Crummock and Ennerdale water sources where Membrane Filtration was installed, the incidence rate ratio for sporadic cryptosporidiosis post compared to pre Membrane Filtration period was estimated to be 0.156 (95% C1 0.076 to 0.320). This meant that the introduction of Membrane Filters was associated with a reduction in incidence of 85.5% (95% C1 68.8% to 92.4%) in Ennerdale and Crummock; whereas in the "Other" water zones, the reduction over the same interval was 61.3% (95% C1: 24.1% to 80.3%), a notable difference.

Risk factors

The interaction term for the risk per pint of cold unboiled tap water consumed each day by the five time intervals of the study adjusted for the other variables in the penultimate model was not significant (p=0.12). The interaction term was dropped in the final model. Drinking unboiled tap water was again shown to be significantly associated with sporadic cryptosporidiosis overall [Odds ratio 1.543 (1.212,1.965)(p for trend<0.001)]. Risk was not significantly different between the different water supply zones. The risk was also increased by contact with cow and other farms [Odds ratio 4.532 (1.757,11.690) (p=0.002)], and feeding pets leftovers [Odds ratio 3.746 (1.214,11.560)(p=0.021)]. A protective effect was seen for consumption of mixed salad [Odds ratio 0.498(0.266,0.932) (p=0.024)]. Most of these impacts were derived from the larger number of cases in the pre intervention period.

No association was detected with age, sex, sewage system of the house, contact with another person outside the household with diarrhoea or with feeding pets biscuits.

Microbiological Results

The *Cryptosporidium* Reference Unit (CRU) confirmed all the isolates of the study cases sent to their Laboratory as *Cryptosporidium parvum.* Over the five years 1998 to 2002, the Reference Unit received on average 68% of the isolates of cases in the study. Analysis of 84 isolates received by the Reference Unit from January 1998 to August 2002, showed 70 isolates (83%) were genotype 2, four (5%) were genotype 1, and 10 (12%) could not be definitively typed. *Cryptosporidium parvum* genotype 2, suggests mammalian (animals and humans) origins, and genotype 1 signifies human origins. The result of the predominantly genotype 2 in these isolates strongly suggests "zoonotic" transmission. This obviously has implications for preventative measures, in terms of drainage into reservoirs, contact with grazing livestock and other animals in the vicinity.

Discussion

The main features of the study highlighted in the above Results Section are discussed extensively in the main text of the Report. The "Discussion sections" have not been included in this Executive Summary as they contain detailed comparisons with other studies, and discursive exploration of the literature on pertinent topics. Readers interested, should refer to the relevant sections of the Main Report, under Sporadic case-control Study - Discussion; and Intervention Study - Discussion.

Conclusions

Sporadic case-control Study

This protracted four year longitudinal case control study in two rural communities in North Cumbria has identified important risk factors of sporadic cryptosporidiosis.

The results showed a highly significant association (p=0.001) between illness in cases and drinking unboiled tap water in all the water supply zones in the local authorities of Allerdale and Copeland in North Cumbria. A dose-response relationship was also found, with the odds of illness increasing by approximately 40% with each extra pint of unboiled water consumed daily. The risk of *Cryptosporidium* infection through consuming water in the different water supply zones was the same, irrespective of whether the water supply zones received water that was unfiltered, a variety of conventionally treated and unfiltered or private.

There were also significant associations between cases and short farm visits, contact with farms, farm animals, animal faeces, any other contact with farm animals, stroking animals, and frequency of farm visits in the single variable analysis. Short farm visits remained significant in the multi-variable analysis (p=0.04), as well as frequency of farm contact (p=0.02).

Eating food within 1-2 hours of contact with a farm animal was found to be a significant risk factor in the single variable analysis. Washing hands before eating, after visits to farms appears to protect against the infection in the multivariable analysis which excluded individuals with mixed water supply.

Microbiological analysis of isolates of cases showed that they were predominantly *Cryptosporidium parvum* genotype 2, the strain with a wide host range, including farm animals.

Several public health messages concerning cryptosporidiosis should be re-emphasised, such as washing hands before eating food, after contact with farm animals; and "boiling potable water", the advice given by the Chief Medical Officer to doctors for the immune-compromised.

Intervention Study

This study examined the impact of the installation of membrane filters on the incidence of *Cryptosporidium* infection in two water supply zones (Ennerdale and Crummock), and compared the results with the "other" water supply zones that did not experience change in North Cumbria. Risk factors were also explored.

The results show that in the post filtration period of this study there was a very clear reduction in the incidence of sporadic cryptosporidiosis in the whole study area. The most likely cause of this reduction was the FMD epidemic and the attendant control and containment measures adopted. This would have applied equally to all the different water supply zones. However, there was a greater reduction in the incidence rates within the Crummock and Ennerdale water supply zones that had new membrane filters compared with the other water supply zones that did not. Thus, despite the confounding effect of the FMD epidemic, the statistically significant differential decrease in the incidence rates of sporadic cryptosporidiosis in these particular water zones could be attributed to the installation of Membrane Filters.

Drinking unboiled tap water remained a

highly significant risk factor overall (p<0.001). Risk was not significantly different between the water supply zones. Risk was also increased by contact with cow and other farms (p=0.002) and feeding pets leftovers (p=0.021).

Microbiological analysis on the isolates for the whole study period, consistently showed *Cryptosporidium parvum* genotype 2 to be predominant for the whole of the study period. The differential dominance of genotype 2 (animals and humans strain) over genotype 1 (the human strain) suggests zoonotic transmission.