# Outbreak of cryptosporidiosis associated with a disinfected groundwater supply

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#### SUMMARY

In an outbreak of cryptosporidiosis in Warrington, a town in North-West England, 47 cases were recorded between November 1992 and February 1993. most within the first month. There was a strong statistical association between cases and residence in an area supplied from two groundwater sources. In a casecontrol study, a strong association between having drunk unboiled tap water from these sources, and a dose-response relationship were found. Occysts were not detected in the water supply. During very heavy rainfall one source of water was found to drain surface water directly from a field containing livestock faeces, thereby bypassing natural sandstone filtration. Exceptionally heavy rainfall occurred at the probable time of infection. After withdrawal of the original water supply, the outbreak rapidly subsided. It was concluded that there was very strong evidence that this outbreak was waterborne. This, the second documented outbreak of cryptosporidiosis attributable to a groundwater supply, demonstrates that infection can be transmitted from a disinfected groundwater source despite apparently satisfactory treated water quality. We recommend that guidelines for protection of groundwater are implemented, raw groundwater should be routinely monitored for microbiological contamination, and the structure of all sources and waterworks should be assessed in risk surveys of water catchment areas.

#### INTRODUCTION

Cryptosporidiosis, which was first recognized to infect humans in 1976, usually causes a self-limiting diarrhoeal illness that resolves in 2–3 weeks. The epidemiology of cryptosporidiosis has been reviewed [1–3].

Waterborne cryptosporidiosis was first reported in Texas in 1984, in a public groundwater source contaminated with sewage by an unknown mechanism [4]. The first confirmed waterborne outbreak in the UK was traced to contamination of a water tank from a cross-connection with a land drain receiving water run off

from an agricultural field [5]. Five hundred confirmed cases in another UK outbreak [6] led to a public expert inquiry into the problem of waterborne cryptosporidiosis [7]. A waterborne outbreak in Milwaukee in spring 1993 was estimated to have infected 400000 individuals [8]. Despite intensive research there are still many unsolved problems in the prevention of waterborne cryptosporidiosis because oocysts are ubiquitous, infectious to many mammals, long-lived, hardy. resistant to chlorination, and may be excreted in very large numbers [9, 10].

This paper reports the investigation and control of an outbreak of cryptosporidiosis associated with a public groundwater source in Warrington, a town in North-West England on the banks of the River Mersey 15 miles from Liverpool. It has a mixed industrial base, a rural hinterland, and is at the crossroads of major transport routes. The first alert was when three cases of cryptosporidiosis were detected on 10 December 1992. By 14 December ten geographically clustered cases had been identified.

A multidisciplinary Outbreak Control Team (OCT) was formed and met on 15 December, when 16 cases were known. Most cases appeared to occur in water supply zone-129 (zone-129), an urban part of North Warrington and two outlying rural villages. Zone-129 received drinking water from one service reservoir supplied from two groundwater sources. On the evening of 16 December zone-129 was switched to a filtered river water supply. A 'boil water' notice was not issued because the change of water supply removed any risk of infection more rapidly, and because of potential adverse effects [11].

# METHODS OF INVESTIGATION

Cryptosporidium oocysts were detected in faecal samples by the modified Ziehl-Neelsen method [12] at Warrington District General Hospital Microbiology Laboratory. Once the outbreak had been recognized, all faecal samples from patients with diarrhoea underwent concentration to improve sensitivity (Evergreen faecal parasite concentrator, Los Angeles). The validity of the diagnosis was confirmed in three cases by the Cryptosporidium Reference Laboratory at Rhyl.

## Collection of data

A case was defined as a patient who had an onset of illness between 15 November 1992 and 28 February 1993, was permanently or temporarily resident in Warrington when their illness began or could have been incubating, and had Cryptosporidium oocysts identified in a faecal sample. Cases were defined as primary if they had no history of contact with a person with diarrhoea in the 3-weeks before the onset of their illness, or otherwise secondary. Local general medical practitioners, medical microbiology laboratories in England and Wales, and consultants in communicable disease control were requested to inform the outbreak team of possibly connected cases.

In six primary cases, a trawling questionnaire, adapted from that used by the Communicable Disease Surveillance Centre, London, was used to enquire about exposure to more than 20 potential risk factors in the 2 weeks prior to the onset of illness. Factors to which at least one patient reported exposure were included in the questionnaire used in a case-control study, namely, spent a night away from

home; eaten cold meats, pies, quiches, raw uncooked vegetables, or yoghurt; drunk pasteurized milk; drunk unboiled tap water, and if so how many cups a day on average. The brand name, date and place of purchase of foods eaten was also sought.

Only primary cases with an onset of illness before 24 December were included in the case-control study, because most cases had an onset before this date and the later the onset the greater the likelihood the case was secondary. Twenty-nine primary cases were compared to two control groups. Two neighbourhood controls were found for each case, and matched for sex, age (within 1 year if < 12 years, otherwise within 3 years), water supply zone of main residence, and residence within 1 mile. A laboratory control was found for 20 cases, and they were matched for age, sex and a microbiologically negative faecal specimen submitted for the investigation of an acute diarrhoeal illness. Three separate case-control analyses were carried out: (1) a comparison of cases and the neighbourhood control group: (2) a comparison of cases and the laboratory control group: and (3) a comparison of cases and a combination of both control groups.

# Data analysis

Conditional logistic regression models which allowed for matching were fitted in the statistical package GLIM [13]. Firstly, each factor was studied individually to find out if there was any evidence of an effect. Secondly, factors which had a probability of less than 0·3 of having no effect were included in multivariable models and were studied by their exclusion, one at a time, from the models. Factors which individually have probabilities of no effect of greater than 0·3 are very unlikely to become significant in a multivariable-model, and so were excluded from further analyses [14]. This step also reduced the number of individuals with missing values. Third, to reduce missing values further, only factors which showed a significant effect (P < 0.05) in the multivariable models were included in the final model.

# Environmental investigations

Detailed information about the water treatment plants, the water distribution system, and the hydrogeology were obtained from records of North West Water Ltd, National Rivers Authority and Warrington Borough Council, and from site visits, and specialized physical investigations. Water quality records were available from 1986. From 16 December 1992–16 March 1993, 30 water samples from the treatment works and service reservoir, and a leisure centre (chosen as it was readily accessible), were analysed for the presence of cryptosporidial oocysts by a standard method [15].

## RESULTS

Forty-seven laboratory confirmed cases were recorded between mid-November 1992 and February 1993 compared to six expected. Two were detected only by faecal concentration. Thirty-seven cases were primary, and ten secondary. The outbreak curve was of a propagated epidemic type, which suggests either personto-person transmission or a continuing common source [16] (Fig. 1). The attack rate was much higher in zone-129 compared to adjacent water zones supply (Table 1, Fig. 2).

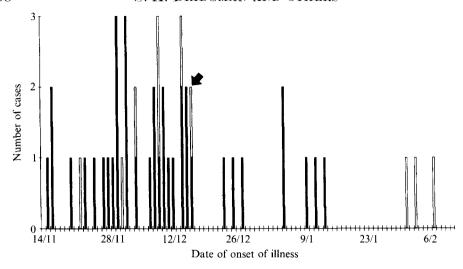


Fig. 1. Primary and secondary cases by onset of illness. The arrow indicates the date (midnight 16 December 1992) that the water supply from Houghton Green and Forest Farm Water Treatment Works was withdrawn. ■. Primary: □. secondary.

Table 1. Crude and age-standardized rates of cryptosporidiosis in this outbreak per 1000 residents by water supply zone

Water		95 %	$_{ m Age\text{-}standardized}$ 95 %		
supply zone*	Crude rate/1000	Confidence intervals	Rate/1000‡	Confidence intervals	
129†	0.81	(0.53-1.10)	0.79	(0.51-1.07)	
130	0.18	(0.06-0.31)	0.18	(0.05-0.30)	
131	0.08	(0.00-0.17)	0.08	$(0.00 \ 0.16)$	
132	0	(0.00-0.18)	0	(0.00-0.18)	
133	0	(0.00-0.13)	. 0	(0.00-0.13)	

<sup>\*</sup> Populations of water supply zones are: 129 = 38252: 130 = 43961: 131 = 38212: 132 = 20582: 133 = 28599.

- † Rates in this water zone are significantly higher.
- ‡ Standard is 1991 Warrington Borough Council population.

A wide age range, very similar to that found in a national survey [17], was observed with a peak in children 0–4 years old (19/47, 40%), and a secondary peak in adults aged 20–29 years (10/47, 21%). There was no association of cases with a particular occupation or institution. In those cases included in the case-control study, all had diarrhoea, 83% (24/29) had abdominal pain, 76% (22/29) nausea, 66% (19/29) vomiting, 62% (18/29) weight loss, and 52% (15/29) fever. In 76% (22/29) illness had subsided by 2 weeks, and in all but one by 30 days: 11% (5/47) were admitted to hospital.

#### Analytical epidemiology

In the neighbourhood control analysis, when individual factors were studied the eating of pies or quiches, the drinking of milk and having spent a night away from home showed no evidence of an effect (P > 0.3) and were therefore not considered

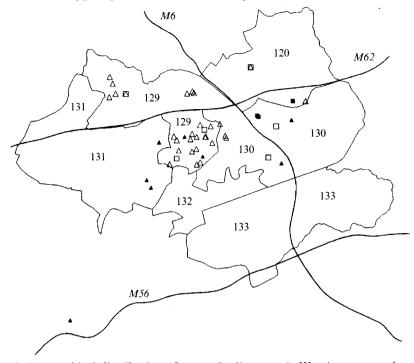


Fig. 2. Geographical distribution of cases. Outline area is Warrington, numbers 120 and 129–133 are water supply zones, and M6, M56 and M62 are motorways. △. Primary early (15 November 1992–16 December 1992); ☐, secondary early (15 November 1992–16 December 1992); ⚠. primary late (17 December 1992–7 February 1993): ☐, secondary late (17 December 1992–7 February 1993).

Table 2. Results of final logistic regression analysis of water consumption\*

Cups of			Prop.	Odds	(95% Confidence
water†	Cases	$NC_{+}^{+}$	cases§	ratio	intervals)
0	4	20	0.17	1.0	(baseline)
1-3	10	25	0.29	4.8	(0.73, 177)
4-6	8	8	0.50	17.5	(2.07.148)
7+	7	5	0.58	28.2	(2.94, 271)

- \* All non-significant variables have been excluded.
- † Average number of cups of unboiled zone-129 tap water drunk per day.
- ‡ NC. neighbourhood controls.
- § Prop cases, proportion of people in each category that were ill.

irther. In the multi-variable model, when each factor in turn was eliminated and he change of deviance calculated, it was found that only the amount of unboiled one-129 tap water drunk had a significant effect on the risk of cryptosporidiosis P=0.025). Of the other factors studied, only the consumption of yoghurt was ose to significance (P=0.05). However, when the amount of unboiled zone-129 he water drunk and yoghurt eaten were looked at together the consumption of oghurt was no longer near significance (P=0.34). Thus in the final model only he drinking of unboiled zone-129 tap water was included and, in contrast to revious models, it had the advantage of no missing values. This final model nowed a highly significant effect of drinking unboiled zone-129 tap water (P=0.00).

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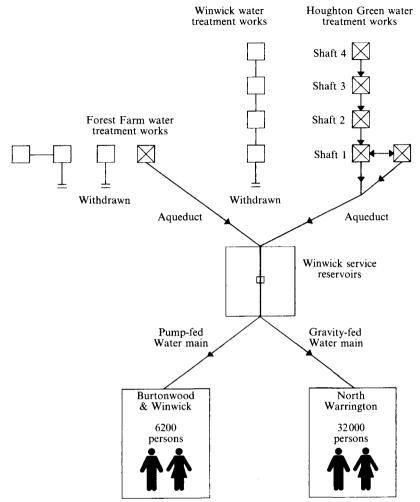


Fig. 3. Schematic plan of water sources and distribution in water supply zone-129 on 15 December 1992. □. Borehole/shaft (deep well): |. headings (link shafts): ⊠. abstraction operational at time of outbreak. Arrowheads indicate flow under active abstraction conditions.

0.003), and the risk of cryptosporidiosis increased (P value for linear trend = 0.0008) as more zone-129 water was drunk (Table 2).

The laboratory control analysis was performed similarly. The only significant risk factors were drinking unboiled tap water and residence in water supply zone-129. In the combined control group analysis, in which both neighbourhood and laboratory controls were considered in one model, only drinking unboiled zone-129 tap water was found to have a significant effect, and there was a positive dose–response relationship.

## Environmental investigations

Groundwater supplied to zone-129 was abstracted from sandstone aquifers at two sites, Houghton Green and Forest Farm. It was then pumped to a single covered service reservoir before distribution (Fig. 3).

Table 3. Rainfall on days with high rainfall around suspected time of waterborne transmission of oocysts and rainfall on 3 January 1994\*

Highest rainfall (mm) during time intervals

	on stated dates							
Date	10 min	1 h	3 h	6 h	24 h			
14 Oct 92	1.8	3.6	5.6	10.2	11.4			
17&18 Oct 92	1.6	7.6	19.6	25.6	35.0			
24 Oct 92	1.6	3.2	7.4	8.4	13.1			
25 Oct 92	1.2	3.8	7.8	14.8	16.5			
24&25 Oct 92					25.6			
27 Oct 92	1.2	4.0	7:0	8.6	12.7			
9 Nov 92	1.8	3.2	5.6	6.4	12.6			
11 Nov 92	1.8	2.8	4.0	6.0	15.1			
16 Nov 92	1.0	2.8	4.4	6.0	11.5			
21 Nov 92	0.6	3.0	5.0	8.8	8.8			
22 Nov 92	0.6	2.6	6.0	9.4	10.9			
30 Nov 92	1.2	3.8	$6\cdot 2$	7.4	17.7			
1 Dec 92	0.6	2.4	2.8	4.0	7.4			
2 Dec 92	5.4	10.2	12.2	15.1	15.1			
11 Dec 92	0.8	$4\cdot 2$	10.2	13.4	17.7			
16 Dec 92	1.8	5.0	6.6	7.2	7.4			
3 Jan 94	1.2	5.2	11.0	16.2	16.7			

<sup>\*</sup> Flooding of Shaft-3 at Houghton Green was recorded on 3 January 1994.

Houghton Green consists of five deep vertical shafts linked by a heading (a horizontal tunnel dug at the base of the shafts to collect and convey water from the surrounding strata). Four of the shafts were sunk at the turn of the century, whilst the fifth was dug in the late 1960s. Although no outcrops of sandstone are visible locally the drift cover is thin or absent with only sandy topsoil obscuring areas shown as outcrops on the 1:50000 drift map [18]. The area was a site of active coal-mining at the time of the outbreak. The amount of subsidence observed made recent fissure-fractures of the sandstone aquifer possible, and these may allow contaminated surface water to bypass normal natural filtration [19]. During exceptionally heavy rainfall on 3 January 1994 Shaft-3 was observed to drain surface water from a flooded field in which livestock grazed; there were similar exceptional days in October to December at the probable time of infection (Table 3. Fig. 4). The inside of Shaft-3 showed evidence of water ingress. An unsuspected cross-connection between a septic tank and unsuspected chambers adjacent to Shaft-1 was found, and Shaft-1 lining had exposed aggregate consistent with corrosion by aggressive waters.

Forest Farm consists of four shafts dug in 1954 and 1955, two of which were linked by a very short heading, and a fifth shaft dug in 1965. It was at much lower risk of pollution than Houghton Green as its aquifer is covered by a 15 metre deep layer of very low permeability boulder clay; is separated from Houghton Green by a major geological fault; is not subject to very recent coal-mining; and its underground workings are much more compact and thus less likely to encounter subsidence or naturally induced fissures.

Cryptosporidial oocysts were not found in any of the water filtrate samples examined at Houghton Green. Down the hole chlorination was performed at both

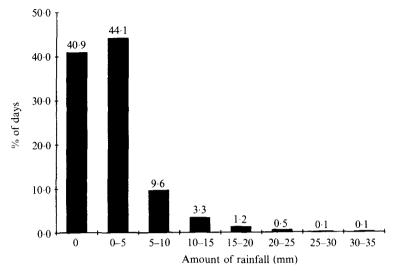


Fig. 4. Frequency distribution of daily rainfall in Warrington. 1988-93.

sources. No raw water monitoring was carried out at Houghton Green. Eleven raw water samples were taken at Forest Farm during 1991 and 1992, all of which were negative for faecal indicator bacteria. Since 1 January 1986 there had only been four coliform failures in about a thousand disinfected water samples from various sites in zone-129, although the only failure in 6 years of samples at Houghton Green occurred in November 1992.

Routine turbidity testing of all three sites generally revealed low values up to 0·3 Nephelometric Turbidity Units (NTU). However, on 4 November 1992 the turbidity was 2·8 NTU at the distribution reservoir, compared to the previous highest value of 1·1 recorded at that reservoir on 28 December 1990. The reservoir inflow had deposits of manganese sediment.

#### DISCUSSION

Of the known vehicles of transmission of cryptosporidiosis, the only one implicated in this outbreak was unboiled tap-water. The strength of the evidence behind the assertion that this outbreak was waterborne is evaluated below by the widely used criteria propounded by Hill [20].

A strong significant association is more likely to represent a cause and effect relationship than a weak one. The geographical distribution of cases showed a very strong association with zone-129 (Table 1, Fig. 1), which is highly unlikely to be a chance finding. This strong association was observed despite the inclusion of several cases that were probably sporadic, and which would tend to bias against finding a true association. If only the primary cases which occurred in the first month of the outbreak are considered then the association of cryptosporidiosis with zone-129 is even stronger. Zone-129 includes two semi-rural outlying villages and an urban area of North Warrington. No other plausible explanation other than their common water supply could be found to explain this unusual geographical distribution of cases.

In the case-control study, a strong association was found between cryptosporidiosis and drinking unboiled zone-129 tap-water (Table 2). No significant association was found with other well-recognized modes of transmission such as food, travel or through an institution [2, 21]. Evidence of causality is strengthened if risk increases as exposure to the suspected risk factor increases [20]. The neighbourhood and all controls analyses demonstrated an increased risk of illness as more unboiled zone-129 tap water was drunk (Table 2). If risk decreases following the removal of a factor, then a causal relationship is more likely. The source of water to zone-129 was changed on 16 December after which there was a rapid decrease in the number of new cases (Fig. 4), which suggests that the outbreak was waterborne and that primary transmission of infection was interrupted.

A causal relationship should be consistent with current knowledge about the distribution and underlying biological mechanisms of the disease [20]. Waterborne transmission is well established for cryptosporidiosis [7]. Worldwide there had been one previously published report of a cryptosporidiosis outbreak attributed to groundwater transmission [4], while recent research in the UK has detected oocysts in several UK groundwater sources without an associated increase in human disease [22].

The plausibility of waterborne transmission is increased by finding environmental mechanisms that can explain the outbreak. Houghton Green Shaft-3 was the most plausible route of entry of pollution as during very heavy rainfall it drained surface flood water from a field which contained livestock faeces, and there was exceptionally high rainfall at the likely time of oocyst transmission. Another possible mechanism included seepage of foul sewage into Houghton Green Shaft-1 from a cross-connection.

Detection of oocysts and/or faecal coliforms in water samples would have increased the plausibility of a waterborne infection. However, negative microbiology is compatible with a waterborne outbreak for the following reasons. Firstly, pathogens are likely to be in low concentrations after dilution in water, and may have passed through by the time an outbreak is recognized; in a review of 672 waterborne outbreaks of disease in the USA between 1946 and 1980, Lippy and Waltrip [23] noted that the causative microbiological agent was isolated from the water system in less than 1% of outbreaks. Secondly, no pre-chlorination (raw) water samples were tested at Houghton Green, and as faecal indicator bacteria are inactivated by adequate chlorination [24, 25], their absence in treated water does not exclude transmission of chlorine resistant organisms like Cryptosporidium [26]. Thirdly, it is so difficult to find oocysts that negative environmental water samples must be interpreted as 'not detected' rather than 'not present' [7]. Fourthly, the infective dose of Cryptosporidium is very small [27].

In the absence of raw water results, the exceptional finding in November 1992 of a positive total coliform culture in disinfected water at Houghton Green provides weak supportive evidence for faecal contamination of raw water, although it could have arisen from other organic contamination. Also suspicious was the exceptional turbidity reading at the service reservoir on 4 November. While a turbidity of 2.8 NTU at the service reservoir is within the maximum

permitted level of 4·0 NTU [28], it was exceptional for this site and therefore suspicious. Increased turbidity levels were associated with waterborne outbrea of cryptosporidiosis in the USA [8, 29]. This outbreak occurred despite treat water meeting regulatory standards, and is a reminder that 'No source of supparan be regarded as free from risk of pollution' [30]. While the UK public have fraccess to information about the quality of treated water supplies it has long be recognized that this information alone may be falsely reassuring [30]. Regroundwater should be routinely monitored for microbiological contaminational though there is no statutory requirement to do so [24, 28].

The National Rivers Authority is currently implementing their policy to def zones of pollution risk around certain points of groundwater abstracti including many water supply sources [31]. The water companies are strong advised to use such zones to help them prevent pollution of their water source We found that archival and field study revealed unsuspected and potentia hazardous cross-connections and structures other than those known by curre staff. Therefore we recommend that the water companies should record elements of existing structures for all their sources by desk study of current a archival records and by periodic direct inspection. This information should made available to other organizations responsible for the protection of the put health, and we believe that this approach should lead to improved record keepi and safety. As recognized waterborne outbreaks associated with groundwater very rare in the UK, guidelines for the protection of groundwater which suggest regular inspection of shafts, etc [30] had not been followed. If they had t outbreak may not have occurred. Therefore we recommend that all UK wa undertakers reassess their preventive strategies to minimize the risk from Cryptosporidium and other parasites.

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