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An outbreak of cryptosporidiosis in South London: what value the *p* value?

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SUMMARY

An outbreak of cryptosporidiosis which affected 44 people in January and February 1991 was identified through local surveillance at a South London Public Health Laboratory. Preliminary enquiries revealed that more than half the patients were adult and that there were no common factors other than geographical association. A case-control study showed a significant association between illness and consumption of tap water supplied by a particular water company, as well as a dose response effect. There were no apparent breaches or irregularities in the water distribution system and no indication of a problem through routine monitoring indices. This incident demonstrates the problems of establishing the source of cryptosporidiosis outbreaks in the absence of evidence of environmental abnormality, as well as possibly indicating that water conforming to current treatment standards may occasionally contain sufficient numbers of *Cryptosporidium* oocysts to cause sporadic cases or clusters.

INTRODUCTION

During the first few months of 1991 a rise in laboratory identifications of *Cryptosporidium* sp was seen in a Public Health Laboratory (PHL) in South London. There were 20 positive identifications between February and March 1991 compared to the expected 1–3 per month.

During April 1991 a further 20 cases of cryptosporidiosis were diagnosed. A preliminary review of 10 cases revealed no common risk factor among cases apart from geographical association and the fact that patients were mostly adult. The extent of the outbreak was assessed by contacting laboratories and Consultants in Communicable Disease Control (CCDC) in or close to the boundaries of the geographic area. The PHL was situated within the boundaries of a Family Health Service Authority (FHSA) which was coterminous with three London Boroughs. Case finding identified a further four cases living within this area.

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The three London Boroughs were supplied by two separate water companies (A and B). Residents of two Boroughs received their domestic water supply mainly from Company A while a large part of the third was supplied by Company B. The majority of cases lived in the areas supplied by Company A.

Because of the observed preponderance of adult cases and their geographical clustering, a case-control study was instigated to test a number of hypotheses including one that drinking potable water from Company A was associated with illness.

METHODS

Case definition

A primary case was defined as any person resident within the geographic area of the FHSA who had suffered gastroenteritis and in whose stool oocysts of *Cryptosporidium* sp had been identified between 27 January 1991 and 15 April 1991. Persons as above, who had had household contact with a person with diarrhoea since 1 January 1991 were defined as secondary cases.

Case finding

Cases were found by contacting Public Health Laboratory Service (PHLS) and National Health Service (NHS) laboratories that served this and surrounding areas, as well as CCDC in the same areas in order to obtain names and addresses of cases that fulfilled the case definition.

Case-control study

Preliminary enquiries suggested no common factors other than geographical clustering. Following these enquiries a questionnaire was designed for use in a postal case-control study. The case questionnaire enquired about the month before illness for consumption of milk and certain food products (raw vegetables, salads, sausages or sausage meat), contact with farm animals or pets, swimming, drinking untreated water and details of nights spent away from home, and enquired about usual daily consumption of potable water, as well as the water company to whom water rates were paid. Questions on water consumption requested data on volume, where consumed and whether the water was boiled. The control questionnaire requested similar information, but enquired about the time period after 1 January 1991.

Names and addresses of controls were supplied by the local FHSA. Random selection of controls was stratified into two age groups: < 10 years and ≥ 10 years. This stratification corresponded to the median age of the patients. As the response rate to a postal questionnaire was anticipated to be 50% or less and controls that had suffered a diarrhoeal illness since the beginning of January were to be excluded, questionnaires were sent to 300 controls to ensure an adequate response.

The FHSA included an explanatory letter to all General Practitioners (GP) in their weekly mailshot giving information about the study, and offering the GP the opportunity to object to their patients being invited to participate in the study. Questionnaires were sent to 37 of 44 identified cases. Seven cases were excluded because three lived outside the defined study area, and four had insufficient or missing addresses. Non-responders were followed up with a letter and subsequently with personal visits.

Cases were excluded from the case-control analysis if they had travelled abroad in the month before onset or if a definite onset date could not be ascertained. Controls were excluded if they had suffered a diarrhoeal illness since 1 January 1991. The questionnaire data were computer entered, verified, and analysed using Epi-info 5 and GLIM software.

Logistic regression, which controlled for age and sex, was used to assess the effects of the various risk factors, separately and combined. The variables in the smallest model adequate to represent the variation in the data were age, sex, water company, the amount of water consumed, and attendance at parties.

Companies A and B were provided with a confidential list of addresses and postcodes of cases and controls so that they could study water supplies to the households, confirm that they were supplied by their company, and investigate any common distribution systems among them. Information about the results of water quality monitoring in the period late 1990 and early 1991 was requested. Detailed information about water supply, treatment and distribution systems, turbidity levels, and results of microbiological testing, information about pollution incidents and any evidence of cryptosporidium oocysts in raw water sources was requested.

RESULTS

Questionnaires from 31 (84%) cases and 112 (37%) controls were returned. Six non-responding patients included one person who probably acquired their illness abroad (Mexico), one patient deceased (from unrelated causes) and two who refused to take part in the study.

Among the 31 patients, there were 15 primary cases and 9 attributed to secondary household contact. Of the 7 remaining, 3 had travelled abroad in the month before illness (destinations were Kenya, Tanzania, Norway and India), 1 questionnaire had been completed by a wrong family member, one questionnaire was returned uncompleted, 1 case had not suffered any gastrointestinal symptoms, and 1 case gave no onset date. These 7 cases and 9 secondary cases were excluded from the analytical case-control study analysis.

Eighty-one of the 112 returned control questionnaires were included in the case-control analysis. Twenty-one controls were excluded because of diarrhoea between 1 January and the time of completing the questionnaire, 10 further questionnaires were inadequately completed.

The following descriptive analysis includes data from primary ($n = 15$) and secondary case questionnaires ($n = 9$). Approximately half of the primary and secondary cases were aged 0–4 years (Table 1). Thirteen cases were male and 11 female compared to 35 male and 46 female controls. Symptoms were typical of cryptosporidiosis [1] with a duration of 2–28 days. Six patients required hospital admission.

Case-control study

Data from 15 primary cases and 81 controls were included in the analytical study. All primary cases lived in the part of the FHSA supplied by Company A.

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Table 1. *Age distribution of cases (n = 24) and controls in South London cryptosporidiosis outbreak*

Age (years)	Primary cases	Secondary cases	Controls
0–4	7	5	36
5–9	1	0	16
10–14	0	0	0
15–24	2	2	2
25–44	4	2	8
45–64	0	0	8
65+	1	0	11
Total	15	9	81

Table 2. *Single variable analysis of risk factors associated with illness in South London cryptosporidiosis outbreak case-control study (n = 96)*

Risk factor exposed	Case		Control		P-Value (Fisher's exact Test)
	Yes	No	Yes	No	
Water supplied by Company A	15	0	57	24	0·018*
Unpasteurized milk	0	14	0	79	—
Contact sick pets	4	10	18	60	0·8844
Contact farm animals	3	11	14	64	1·00
Use water filter	2	12	7	72	0·812
Attend party	1	10	40	37	0·0144*†
Swimming	7	7	34	47	0·783
Drink untreated water	1	13	1	78	0·560
Contact water outdoors	0	15	10	69	0·318

* Significant association.
† Apparent protective effect.

No cases lived in the area supplied by Company B. Seventy per cent of the controls lived in the area supplied by Company A and 30% in the area supplied by Company B.

Logistic regression did not show age to be significantly associated with illness and the following analyses were not stratified for age. There was no association between illness and drinking unpasteurized milk, contact with lakes or streams, contact with sick pets, farm animals, or swimming in the month before illness. Regular consumption of certain foods including raw vegetables, salads, sausages or sausage meat was not significantly associated with an increased risk of illness. Three cases reported using a drinking water filter at the time of interview, but none of them had used a filter regularly. There were positive associations with water consumption and negative associations with party attendance.

The following were the main findings of the study:

(i) Living in a household supplied by Company A compared to a household supplied by Company B was significantly associated with illness, OR undefined; ($P = 0{\cdot}018$) (Table 2).

(ii) Attending parties or functions appeared to have a protective effect, OR = 0·09; ($P = 0{\cdot}014$).

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Table 3. *Usual daily consumption of water among cases and controls resident in area supplied by Company A (n = 72) South London cryptosporidiosis outbreak*

	More than 1 glass	Up to and including 1 glass	Total
Cases	12	3	15
Control	26	31	57
Total	38	34	72

Odds Ratio = 4·77, 95% CI 1·1–28·5, $P = 0·037$.

Table 4. *Dose Response to Company A water consumed among cases and controls in area supplied by Company A (n = 72) South London cryptosporidiosis outbreak*

	None	Up to 3 glasses	More than 3 glasses	Total
Cases	2	6	7	15
Controls	24	18	14	56
Total	26	24	21	71*

χ^2 for trend = 4·65, $P = 0·031$.
* Volume data for one control were incomplete.

(iii) In an analysis restricted to cases and controls resident in the area supplied by Company A ($n = 72$), consumption of more than one glass of water a day at home was significantly associated with illness, OR = 4·77; ($P = 0·037$) (Table 3).

(iv) A dose-response relationship between increasing tapwater consumption and illness was also demonstrated among cases and controls living in the area supplied by Company A. ($P = 0·031$) (Table 4).

Logistic regression analysis restricted to cases and controls from the Company A supply area showed that risk of illness was associated with water consumed at home ($P < 0·001$) and partygoing no longer was a significant factor associated with decreased risk.

Environmental and microbiological results

Addresses of the 15 primary cases fell into 7 different water supply zones and 5 different pressure zones of Company A. There were no common links between cases and a single supply zone or group of related zones or a trunk main or a service reservoir. A breach of integrity of the system was considered to be unlikely. Three major treatment works were supplying these zones around the time of onset of symptoms. The raw water to these works originated from two impounding reservoirs (R1 and R2) and water was abstracted from the same river into these reservoirs.

Most of the cases were supplied by a number of direct pumping mains from one or more treatment works. No operational irregularities at any of the three treatment works were identified. No pollution events or serious agricultural incidents such as animal slurry spills in the catchment area were reported by the National Rivers Authority (NRA) or Ministry of Agriculture, Fisheries and Food (MAFF). Weekly sampling of raw source waters was carried out at the highest abstraction points, and weekly sampling at the abstraction point to R1 and R2 was also carried out.

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Data provided by Company A revealed that since monitoring began in 1989, cryptosporidium oocysts were found on only five occasions in raw source water. The number of oocysts in these positive samples was low, not exceeding four oocysts per litre. Operational monitoring of filter backwash water at a treatment works where source water was partially from the same source river was also considered unremarkable with just one positive sample in January 1991. When storage in impounding reservoirs was taken into consideration, the timing of these positive findings did not seem to correlate with the time of onset of symptoms in cases.

All three source works employed slow sand filtration, and dual filtration (rapid primary filters followed by slow sand filtration) was used at two of the works. Raw water was taken from the river into storage reservoirs with an average holding time of 30 days (range 10–60 days) prior to treatment. Disinfection was applied after filtration at each works.

There were no operational problems at these works and by-passing of filters was reported not to have occurred. Slow sand filter quality was monitored routinely and return of filters to service after cleaning or resanding was subject to procedures which included monitoring and running to waste as specified in the Badenoch report [2]. Coagulants were not used in treatment processes at these works. Backwashing and performance of primary filters was monitored in terms of particle removal, turbidity, particulate organic carbon and other relevant parameters which were reported to have been unremarkable.

Treated water leaving the works, service reservoirs and customers' taps were stated to have been monitored in accordance with the drinking water regulations. Coliforms were not present in any samples collected from the area during the time in question and no abnormal results were recorded for other parameters. Residual chlorine levels were within the expected range and levels leaving the works were reported to be higher than in previous Winter/Spring seasons. The service reservoirs did not have any known structural defects and there was no indication from monitoring of any water quality problems.

DISCUSSION

Cryptosporidiosis is an important cause of diarrhoea in the immunocompetent as well as in immunocompromised patients [3, 4]. Between 1986–9 laboratory reports of faecal identifications of cryptosporidium oocysts to the Public Health Laboratory Service (PHLS) Communicable Disease Surveillance Centre (CDSC) increased sharply from 2750 to 7768, and then fell to 4680 in 1990, rising again to 5165 in 1991 (PHLS Communicable Disease Surveillance Centre, unpublished data).

Person-to-person spread is thought to be responsible for many cases especially in young children [1], and exposure to farm animals and drinking raw milk have also been shown to be associated with infection [1, 5]. These were not found to be risk factors in this South London outbreak where a statistical association was found between illness and drinking tap water. After allowing for the possible confounding effects of exposure to other recognized risk factors, the epidemiological evidence suggested an association between infection and the consumption of water supplied by Company A. The evidence that the outbreak was waterborne was epidemiological without positive microbiological findings and the results of raw water monitoring did not provide any evidence to support the hypothesis that potable water was contaminated. The epidemiological evidence from the case-control study gave two significant associations, drinking water from supply A and a dose-response relationship. The vehicle of infection is highly plausible as previous outbreaks of cryptosporidiosis have implicated water supplies and waterborne transmission has been well documented in the UK [6] as well as recently in an outbreak affecting an estimated 403000 people in the United States of America [7].

Even if there is routine monitoring the chances of sampling at the right place and time are slim and detection methods are not perfect. Water treatment processes including filtration and chlorination cannot be relied upon to prevent all oocysts from entering mains water but they are usually effective in dealing with low numbers of the parasite [8]. Previous studies have shown that water monitoring indices may fail to demonstrate a potential risk to human health [2, 8, 9], that the established methods for detecting oocysts may vastly underestimate the number present in water [10], and that the minimum infective dose may also be very low [4, 8]. More important from the public health viewpoint is the inability of tests used to distinguish between different species of oocysts or determine whether the oocysts are viable [8]. New techniques to enable such discrimination are being developed [11]. Indeed in a Scottish study using Restriction Fragment Length Polymorphism (RFLP) techniques, viable oocysts were found rarely in the particular water source studied and their presence was not associated with an increased risk to health [12]. The detection of oocysts in raw water is of itself not unexpected and studies have shown oocysts in 7·8% samples examined [13].

Slow sand filtration has been shown to be the most effective way of removing oocysts from raw waters and was in use at all three treatment works. Documented waterborne outbreaks have been attributed to operational irregularities enabling oocysts to bypass treatment steps [14], and in this outbreak, it was noteworthy that no documented operational or monitoring irregularities were reported to have occurred. Oocyst breakthrough of water treatment, if it occurred, was presumably at a very low and probably undetectable level.

Thus we were faced with a plausible epidemiological association and a dose response effect unsupported by corroborating microbiological or other evidence of a problem with water supply.

Our epidemiological study can be criticized as the control response rate was low and the interval from the time of illness to investigation in some cases was longer than 2 months thus recall bias may have been introduced. In addition, our case study size was small, and represented an attack rate of approximately 1 per 10000 population. The outbreak was recognized in a PHLS laboratory which screens all stools for *Cryptosporidium* and it is possible that the extent of the outbreak in London may not have been fully identified as London-wide monitoring for *Cryptosporidium* is inconsistent; some laboratories do not screen for *Cryptosporidium* and laboratories that do screen have differing screening and reporting policies.

The PHLS has instituted a policy throughout its network of laboratories that all stools from children and adults aged up to 44 years with presumptive infectious diarrhoea should be tested for *Cryptosporidium* oocysts as a minimum and testing of all loose stools is considered a sensible approach. Such standardization of laboratory policy is desirable and also has been recommended in National Health Service, and other laboratories [2]. As waterborne outbreaks of cryptosporidiosis in the Thames area would be likely to affect more than one region, a standardized surveillance scheme should ideally be agreed within the area. A prospective study could be based on the findings of such a surveillance scheme. London-wide monitoring is important and has been recommended by the Central London Communicable Disease Control Group. The instigation of this would allow any London-wide problems to be quickly identified and investigated promptly.

In conclusion, this small study showed a statistically significant epidemiological association between infection and consumption of tap water without any supporting evidence of environmental contamination, operational irregularity, or recorded problems with or changes to the water treatment. It was not possible to establish a causal relationship. The epidemiological evidence was cumulative. Other potential contributory factors were enquired about in the study and found not to be associated. The association with tap water consumption was fairly strong, and most importantly demonstrated a clear dose response effect. Such epidemiological studies without any additional supporting microbiological evidence are difficult to interpret and in order to help us reach firm conclusions in these situations there is a need for better surveillance data together with improved microbiological techniques to confirm the presence of viable cryptosporidium oocysts. The incident clearly demonstrates the importance of microbiological and environmental evidence to support the findings of epidemiological studies which otherwise leave us questioning the value of the P value.

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