A probable waterborne outbreak of cryptosporidiosis in the Sheffield area

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Summary. There was a marked peak in human cases of cryptosporidiosis in the Sheffield area in May and June 1986. Extensive epidemiological investigations failed to find a common source of food or a consistent history of animal contact, but did suggest that a waterborne outbreak of cryptosporidiosis may have occurred. *Cryptosporidium* oocysts were found in untreated water and in fish from a reservoir complex implicated by epidemiological analysis. Laboratory investigations confirmed that cattle on a farm adjacent to the reservoir complex were a possible source of contamination.

Introduction

The coccidian parasite, *Cryptosporidium*, is now recognised as an important intestinal pathogen causing acute diarrhoeal disease in man.¹ The source of infection for man may be diverse. Infections in farm, domestic and laboratory animals are common,^{2–4} and early reports suggested that close contact with animals was the usual source of infection for man.^{5,6} Transmission of *Cryptosporidium* from animals to man *via* food and milk has been documented^{7,8} and person-to-person transmission of *Cryptosporidium* has been reported in hospitals and nurseries.^{9,10} Of major public health concern are reports,^{11,12} supported by epidemiological and environmental evidence, of possible transmission of *Cryptosporidium* *via* contaminated drinking water treated by conventional means to remove bacterial and viral pathogens.

In the Sheffield area, from late April to October 1986, we observed an increase in human cases of cryptosporidiosis, with a marked peak in May and June. No changes in laboratory methods for detecting cryptosporidium oocysts took place at that time. Extensive epidemiological investigations by Sheffield Environmental Health Department (EHD) failed to find a common source of food or milk, or a consistent history of close contact with animals. However, of 62 patients recognised in May or June, 49 (79%) drank water from the same reservoir complex.

The purpose of this study was to determine

whether the hypothesis of a waterborne outbreak of cryptosporidiosis in Sheffield could be supported.

Materials and methods

Selection of samples

Man. All faecal samples from human cases of acute diarrhoea submitted to Sheffield Public Health Laboratory (PHL) between January 1985 and December 1987 were examined for oocysts of *Cryptosporidium* and for other recognised intestinal pathogens.

Cattle. Faecal samples from cattle on a farm adjacent to the implicated reservoir complex were taken by the Veterinary Investigations Centre (VIC), Loughborough, where smears were stained by a modified Ziehl-Neelsen (ZN) method, and examined microscopically.

Fish. Wild brown trout (*Salmo trutta*) were netted from the reservoir complex by the Yorkshire Water Authority (YWA). Intestinal contents were removed into sterile universal containers and examined at Sheffield PHL.

Water. Pre- and post-treatment samples of water (10 or 20 L depending on turbidity) were collected by YWA from the two reservoirs in the complex, feeder streams flowing into the reservoirs, a river flowing out of the reservoirs, and children's paddling pools fed by the latter river. Water samples were examined at Sheffield PHL.

Examination of faecal samples

All human faecal and fish intestinal samples were examined for oocysts of *Cryptosporidium* by microscopic examination of a direct smear stained by a modified ZN method.¹³ Samples containing bodies resembling such oocysts were tested by a direct immunofluorescence (IF) method, with mouse IgM monoclonal antibody (MAb)

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conjugated to fluorescein isothiocyanate (FITC)¹⁴ supplied by the Division of Microbiological Reagents and Quality Control (DMRQC), Central PHL, London.

All human faecal samples were examined also for various other intestinal pathogens by methods described previously.^{15,16} Fish intestinal samples were examined also by an enzyme immunoassay (EIA) for *Cryptosporidium* described below.

Examination of water samples

Water samples were passed through membrane filters (142 mm diameter, 0.45 µm pore size; Pall Process Filtration Ltd) in a Sartorius filtration system (Sartorius, West Germany). Specimens containing much debris were allowed to settle overnight at 4°C before membrane filtration of the supernate. Filters were cut into strips, placed in a sterile jar and shaken vigorously with 100 ml of phosphate-buffered saline pH 7.2 (PBS) containing Tween 80 1% v/v. The suspensions were left overnight at 4°C and centrifuged at 3000 g for 20 min at 10°C. The deposits were then examined for oocysts of *Cryptosporidium* by microscopy as above, and also by an EIA for *Cryptosporidium* as below.

EIA for *Cryptosporidium*

The assay, described fully in another report,¹⁷ was briefly as follows: 96-well polystyrene EIA plates were coated with MAb to *Cryptosporidium*; uncoated sites were blocked by bovine serum proteins; samples of faecal suspension or water deposit were added and any cryptosporidium antigen allowed to react with the MAb; MAb to *Cryptosporidium*, conjugated to FITC, was added to react with the cryptosporidium antigen; MAb against FITC, conjugated to horseradish peroxidase, was added to react with the FITC; peroxidase activity was measured with the chromogenic substrate 3:3',5',5' tetramethylbenzidine. Between stages, plates were washed three times with PBS containing Tween 20 0.5% v/v. All antibodies and conjugates were supplied by DMRQC, and were used at their optimal dilutions.

Statistical analysis

For specimens sent to Sheffield PHL in May and June 1986, χ^2 analysis was used to compare the incidence of cryptosporidiosis with the incidence of all diarrhoeal illness, in patients receiving drinking water from the

Figure. Monthly incidence of cryptosporidiosis in Sheffield.

reservoir complex, and in those receiving water from other sources.

Results

Detection of cryptosporidium oocysts

Man. The figure shows the monthly numbers of cases of cryptosporidiosis from July 1985 to June 1987, with a marked peak in May and June 1986. Analysis of the data from this peak period showed a significant association of cryptosporidiosis with the supply of drinking water from the suspect reservoir complex (table I).

Of 935 human faecal samples examined in May and June 1986, 284 contained a recognised intestinal pathogen; of these, 62 (22%) were *Cryptosporidium*.

Cattle. In a verbal report, the VIC indicated that most of the samples of cattle faeces contained oocysts of *Cryptosporidium*; unfortunately precise figures were not available.

Fish. *Cryptosporidium* oocysts, morphologically indistinguishable from those found in the human and bovine faecal samples, were found in the intestinal contents of apparently healthy brown trout. Of 18 specimens, seven were positive by both microscopy and EIA, two by microscopy alone and five by EIA alone (table II).

Water. Oocysts of *Cryptosporidium* were found in 14 untreated water samples from the two reservoirs, and in water samples from the streams and the river, but were not found in two samples of treated water (table II).

Discussion

The results suggest that there may have been a waterborne outbreak of cryptosporidiosis in Sheffield in May and June 1986. Cattle are a well-documented source of cryptosporidium oocysts,^{3,18} and contamination of the reservoir system from this source most probably occurred from surface water after heavy rainfall. Water treatment that

Table I. Cases of cryptosporidiosis and acute diarrhoea during May and June 1986 in relation to water supply

Patients	Number of patients receiving drinking water from	
	suspect reservoirs	other supply
Confirmed cases of cryptosporidiosis	49*	13
Cases of acute diarrhoea	537	398

* Significant association of cryptosporidiosis with supply from suspect reservoirs ($\chi^2 = 11.51$, $v = 1$, $p < 0.001$).

eliminates bacterial and viral pathogens may allow viable oocysts to survive, so that water implicated in outbreaks of cryptosporidiosis may meet coliform and turbidity standards for drinking water.¹⁹ Therefore, examination for oocysts in water would be a necessary though laborious and costly procedure to minimise the risk of waterborne cryptosporidiosis. Various filtration and separation methods have been described, but oocysts so obtained often have altered morphology and react poorly in conventional and IF staining procedures.²⁰⁻²² Better methods of detecting oocysts are needed.

Reports of *Cryptosporidium* in fish are uncommon.²³ The significance of our finding of cryptosporidium oocysts, morphologically indistinguishable from those found in man, in apparently healthy brown trout, is unclear. It does, however, confirm the widespread presence of the organism in the water catchment system.

We did not find oocysts in two samples of treated water from the reservoir complex in early 1987. This may suggest that routine treatment procedures reduce the level of contamination below the limits of detection of the available methods. However, it

Table II. Detection of *Cryptosporidium* in environmental samples

Nature of specimen (number of samples)	Number of samples in which oocysts were detected by			
	microscopy and EIA	microscopy alone	EIA alone	neither method
Surface water from reservoirs A and B (14)	10	0	4	0
Treated drinking water from reservoir A (1)	0	0	0	1
Treated drinking water from reservoir B (1)	0	0	0	1
Brown trout intestinal contents (18)	7	2	5	4

is possible that a treatment failure may have occurred early in 1986, which was either unnoticed or had been rectified before a waterborne outbreak was suspected and water samples examined for oocysts. The water was examined some 6 months after the peak of the putative outbreak, when the incidence had fallen to the normal endemic rate and there was no association with the water supply.

Although the infective dose for man is not known, ingestion of as few as 10 oocysts may cause cryptosporidiosis in primates.²⁴ Even small numbers of oocysts in drinking water must, therefore, be considered a potential hazard. Oocysts may be reduced in number, but not completely removed, by normal water filtration methods;^{12,20} they are resistant to many disinfectants¹⁸ and probably to

routine chemical treatment, and they may remain viable in water at 4°C for at least 140 days.²⁵

The present findings support the hypothesis, though do not prove, that a waterborne outbreak of cryptosporidiosis occurred in Sheffield during 1986. Increased awareness of the possibility of waterborne spread of *Cryptosporidium*, and better methods for detecting oocysts in environmental samples, would help in the investigation of future outbreaks.

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