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. The source of infection for man may be diverse. Infections in farm, domestic and laboratory animals are common, and early reports suggested that close contact with animals was the usual source of infection for man. Transmission of Cryptosporidium from animals to man via food and milk has been documented and person-to-person transmission of Cryptosporidium has been reported in hospitals and nurseries. Of major public health concern are reports 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. Samples containing bodies resembling such oocysts were tested by a direct immunofluorescence (IF) method, with mouse IgM monoclonal antibody (MAb)
conjugated to fluorescein isothiocyanate (FITC) 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. 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, 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, χ² analysis was used to compare the incidence of cryptosporidiosis with the incidence of all diarrhoeal illness, in patients receiving drinking water from the
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 Shef-
field 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
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 proce-
dure to minimise the risk of waterborne cryptospor-
idiosis. 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 uncom-
mon.23 The significance of our finding of
Cryptosporidium oocysts, morphologically indis-
tinguishable 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 I. Cases of cryptosporidiosis and acute
diarrhoea during May and June 1986 in relation
to water supply

<table>
<thead>
<tr>
<th>Patients</th>
<th>Number of patients receiving drinking water from</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>suspect reservoirs</td>
</tr>
<tr>
<td>Confirmed cases of cryptosporidiosis</td>
<td>49*</td>
</tr>
<tr>
<td>Cases of acute diarrhoea</td>
<td>537</td>
</tr>
</tbody>
</table>

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

Table II. Detection of Cryptosporidium in environmental samples

<table>
<thead>
<tr>
<th>Nature of specimen (number of samples)</th>
<th>Number of samples in which oocysts were detected by</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>microscopy and EIA</td>
</tr>
<tr>
<td>Surface water from reservoirs A and B (14)</td>
<td>10</td>
</tr>
<tr>
<td>Treated drinking water from reservoir A (1)</td>
<td>0</td>
</tr>
<tr>
<td>Treated drinking water from reservoir B (1)</td>
<td>0</td>
</tr>
<tr>
<td>Brown trout intestinal contents (18)</td>
<td>7</td>
</tr>
</tbody>
</table>
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. 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; they are resistant to many disinfectants and probably to routine chemical treatment, and they may remain viable in water at 4°C for at least 140 days.

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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REFERENCES