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Cryptosporidium plus campylobacter: an outbreak in a semi-rural population

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SUMMARY

An outbreak of cryptosporidiosis in a circumscribed semi-rural population is described; some cases were infected concurrently with campylobacter. The results of a detailed case-control study and environmental surveillance are discussed.

INTRODUCTION

Cryptosporidium sp. is an important agent of gastrointestinal disturbance in both animals and man (Editorial, 1984); clinical features are those of a protracted but self-limiting gastroenteritis, and include profuse, foul-smelling watery diarrhoea, abdominal pain, vomiting, anorexia and weight loss. A variable incidence has been reported but in this laboratory oocysts are present in approximately 2% of specimens from all cases of diarrhoea examined. Attention has been drawn to an increased incidence in children (Casemore & Jackson, 1983). Cryptosporidia have been described in a variety of animal species and the infection in humans has therefore been assumed to be a zoonosis, but this can be questioned (Casemore & Jackson, 1984). Outbreaks in nursery-school populations (Alpert *et al.* 1984; Casemore, unpublished data) and urban outbreaks (Hunt *et al.* 1984) have been described. Adult human-to-human transmission occurs (Blagburn & Current, 1983; Baxby, Hart & Taylor, 1983). Thus epidemiological studies of such infections are needed to provide information leading to an informed approach to containment.

During the last 2 weeks of March 1984 four consecutive specimens of faeces received from adult patients living in Holywell, a small town in a predominantly rural area, were found to contain cryptosporidia; the last of these also yielded a campylobacter. In addition to these general-practitioner patients, a child in the paediatric ward of the District General Hospital who lived in an immediately adjacent area was found to be suffering from diarrhoea and excreting cryptosporidia. The assistance of the Communicable Disease Surveillance Centre, Colindale, was sought, together with that of the local Community Health and Environmental Health Departments, in order to investigate these cases. As further cases of

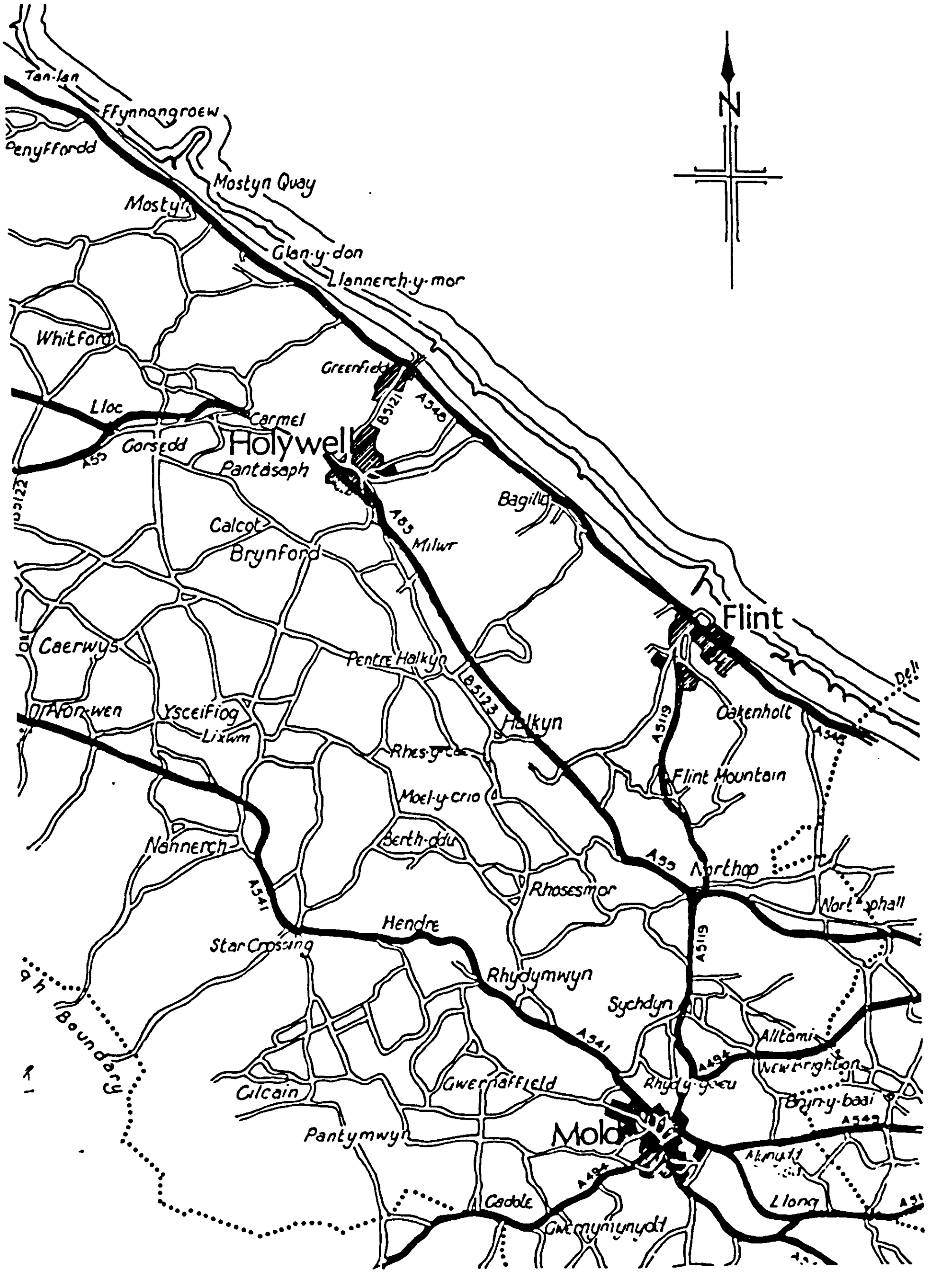

Fig. 1. Legend. Map of area involved.

cryptosporidiosis continued to occur in the area, the possibility of a water, milk or food-borne outbreak was investigated. Salmonella infection from raw milk had occurred in the area on previous occasions.

MATERIALS AND METHODS

Case finding and epidemiological sampling

The definition of a case was anyone, whether symptomatic or not, in whom cryptosporidia were found to be present in the faeces.

The investigation began on 3 April 1984; the five methods of case finding were as follows:

(1) General practitioners were asked to submit faecal specimens from all cases of gastroenteritis,

(2) All hospital admissions with gastroenteritis were identified as to their home locality,

(3) Household contacts of cases were asked to provide faecal specimens,

(4) Local schools were visited so that cases of gastroenteritis could be followed up,

(5) The major industrial employer in the area was asked to participate in the investigation of cases of diarrhoea in the staff.

Serological tests to detect the presence of cryptosporidial antibody were performed, wherever possible, to confirm the infection and also in control groups. The area under surveillance is shown in the map (Fig. 1).

Other cases were discovered in outlying areas and these were found to have either direct or indirect links with Holywell.

The natural history and epidemiology of *Cryptosporidium* (and that of *Campylobacter* sp.) suggested several areas which required investigation and environmental samples were sought from the following:

(i) Two farms in the area that were known to supply milk, the fields of which were adjacent to infected households.

(ii) A 'country park' recreational development consisting of a series of large ponds.

(iii) Milk supplies, including the distribution of both pasteurized and unpasteurized milk.

(iv) Household pets and wildlife.

(v) Housing estates within the area.

(vi) Poultry producers.

(vii) Drains and surface water.

Microbiological studies and methods

The laboratory serves North Wales. It receives environmental samples and specimens from patients of all ages submitted by general practitioners, environmental health officers and hospital medical staff throughout that area.

The methods used to detect cryptosporidia in faeces were those previously described (Casemore, Armstrong & Jackson, 1984). Specimens from family contacts of cases were subjected to preliminary screening and, if negative, were concentrated by a modification of the formol-ether method, which was found to be more sensitive than the generally recommended sucrose method (Casemore, Armstrong & Sands, 1985). Faecal specimens were cultured for a range of bacterial pathogens and viruses and also examined by electron microscopy.

Samples of pet and livestock faeces were examined by the concentration method described above. Streams, ponds and drains were sampled using swabs (Moore, 1948) that were examined after concentration by centrifugation of eluate. Milk filter 'socks' were macerated in a blender, extracted and then concentrated as for faeces. Milk samples were centrifuged and the pooled deposits were similarly extracted and concentrated prior to microscopical examination.

Antibody studies were carried out using purified whole oocysts in a sandwich technique with anti-human IgG, A and M conjugates, absorbed to remove unwanted staining; the detailed method is described elsewhere (Casemore, 1985). In a few cases paired sera were available but in most only a single serum was available. Several categories of control sera were also examined in parallel; for example, those from patients in the same area who had provided specimens for reasons other than gastrointestinal illness, volunteers without illness, potentially high risk groups and those from outside the area in whom cryptosporidia had been detected.

The case-control study

The first ten confirmed positive households were asked to nominate three comparable neighbouring households as controls. Three age bands for positive cases were used: 1–4 years, 5–15 years and 16–70 years. All households were interviewed using a specially constructed questionnaire including:

(1) Personal details and recent medical history (control patients giving a recent history of diarrhoea were excluded).

(2) Foods consumed, both at home and elsewhere.

(3) Consumption of milk including the quantity and type of milk.

(4) Water supply, including the source of water.

(5) Contacts with pets and other animals.

(6) Clinical details where relevant.

Differences between cases and controls were examined using Fisher 's exact test (FET); if there was a suspicion of a significant difference (i.e. FET value $> 0{\cdot}25$), the exact probability for matched samples was evaluated for two-sided probability to allow for protective as well as risk affects.

All subsequent cases that had not been included in the above study were interviewed individually as they arose, without seeking controls and without detailed statistical analysis.

RESULTS

During the year before onset of the outbreak, 2172 specimens of faeces had been examined for cryptosporidia, of which 22 were found to be positive. Most were from children living in town and presenting as sporadic cases. During the 3 months March–May 1984, 642 specimens of faeces yielded 24 positives – a statistically significant increase. Of these 19 lived either in Holywell or had a direct connection with it (Table 1).

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Table I. Cryptosporidium *positive patients (7 adults; 12 children of mean age 4·2 years; 11 M:8F)*

Case no. and initials	Age (years)	Sex	Date of onset (or 1st positive finding)	Notes
1 PR	37	M	10 March	Home adjacent to case 2
2 JB	37	M	12 March	Plumber working on farm
3 SW	36	F	12 March	—
4 SR	4	M	20 March	Admitted Glan Clwyd Hospital 21 March
5 GR	2	M	26 March	Brother of case 4
6 JH	29	M	24 March	Also positive for campylobacter
7 ME	9	M	27 March	—
8 MF	4	M	29 March	Mother and father both had campylobacter alone
9 LF	2	F	29 March	Sister of case 8; also campylobacter positive
10 CH	8/12	F	31 March	Child of cases 6 and 13
11 DT	20/12	M	2 April	—
12 FC	6	F	2 April	Sister of case 14
13 EH	19	F	5 April	Wife of case 6
14 KC	8	F	6 April	Admitted to Glan Clwyd Hospital
15 SM	29	F	6 April	Vomiting but *no* diarrhoea
16 MS	2	M	22 April	—
17 JS	4	M	4 May	Brother of case 16
18 PJ	7	M	7 May	—
19 SI	30	F	18 May	—

All specimens were examined for campylobacter; there were 34 human isolates of which 11 were from patients in the Holywell area. Five of these occurred in members of two families in which *Cryptosporidium* was also present, and in three cases both organisms were present in the same individual. The presence of campylobacters was usually indicated by the presence of pus cells and red blood cells in the specimen; those with cryptosporidium by itself were characteristically cell free. Five individuals in four households were found to be excreting campylobacter alone; household contacts were screened for cryptosporidium with negative results. No other recognized faecal pathogen was found although a variety of such pathogens were present in specimens submitted to this laboratory from patients in other areas.

In one case (14) a specimen of vomit contained cryptosporidia.

Case control study

Examination of laboratory records showed only one detection of *Cryptosporidium* in the Holywell area in the year prior to the outbreak. Campylobacter isolations in North Wales generally follow the national pattern, with a major summer peak followed by a lesser peak in the autumn. In the 2 months before the outbreak there had been only four isolations in the area described.

Most patients with cryptosporidiosis were diagnosed following a consultation with their general practitioner and complained of persistent, offensive, watery diarrhoea, usually with vomiting, anorexia, abdominal pain and weight loss; a few additional positives were found among household contacts and most of these were also symptomatic, though often with a less-severe illness. One patient had vomiting but no diarrhoea. Enquiry among the local schools and the work force of the local major industrial employer failed to reveal further cases.

There was statistically no significant association between illness and the consumption of raw milk. Further detailed enquiry, however, revealed that a high proportion of the local population usually consumed raw farm-bottled milk and that some cases, who normally bought pasteurized milk for their use at home, consumed raw milk when visiting other housholds. Analysis of eating habits for other food items unexpectedly revealed a possible association with consumption of sausage; distinction could not be made as to the type or source of the sausage, the method of cooking or the practice of eating raw sausage, which is known to occur.

There was no evidence to implicate the water supply; all cases and controls used the same mains water. However, environmental contamination with cryptosporidia of the source of the local water supply prior to treatment has been shown to occur by demonstration of oocysts in upland reservoir feeder streams.

No significant difference was detected between cases and controls for exposure to animals.

A curious finding was the preponderance of males, 8 of 10, during the period of the case-control study, although this was subsequently less marked, 11 of 19 overall. The average household size was 3·4 for cases and 3·67 for controls. Person-to-person spread is known to occur and this is especially so among children; all but one of the households investigated had a first-degree relative attending a school in Holywell. Screening of schoolchildren to detect subclinical infection was, however, not attempted.

Environmental and epidemiological studies

The results of examination of samples from pets from ten households (4 dogs, 2 hamsters, 1 budgerigar, 1 aviary of budgerigars and 2 pet lambs) showed that the aviary and lambs (which were dead) yielded cryptosporidia in small and large numbers respectively. The budgerigars kept in an aviary were in a garden belonging to case 16, which was adjacent to fields used for grazing; green weeds were collected from this field for the cage birds. The two bottle-fed pet lambs that belonged to case 14 became ill and died from severe scouring at about the same time. On investigation, her sister (case 12) was found to have been mildly ill with gastroenteritis during the preceding week and was found on examination to be excreting cryptosporidia in large numbers. The lambs were exhumed for examination and bowel contents showed very large numbers of cryptosporidia; bacteriological culture and electron microscopy gave negative results. As this family lived some distance from the area described, it was initially thought to be unconnected with the outbreak. However, the household received a supply of milk from the canteen of the factory in the area of the outbreak; the father of cases 8 and 9, who was found to be excreting campylobacter, was a chef in this canteen.

Case 2, a plumber, had been carrying out work on a farm in Greenfield that

Table 2. *Serological response to* Cryptosporidium

Patient no., initials and stage	Date of specimen (1984)	Titre IgG	IgA	IgM
	Paired sera			
1 PR				
Acute	19 Mar.	5	≤ 5	5
Conval.	27 July	80	20	20
3 SW				
Acute	23 Mar.	10	5	5
Conval.	26 July	40	80	30
14 KC				
Acute	11 Apr.	5	5	10
Conval.	16 May	20	40	80
18 PJ				
Acute	17 May	≤ 5	< 5	< 5
Conval.	7 June	40	20	20
19 SI				
Acute	23 May	< 5	5	10
Conval.	9 Aug.	5	20	80
	Single sera (late)			
2 JB (M)	22 Aug.	20	10	20
7 ME	26 July	30	30	30
15 SM	23 July	< 5	10	20
17 JS	24 July	20	10	20
	Case from control group			
20 JB (F)	15 Aug.	5	160	20
	1st degree family contacts (case no.)			
(1) DR	27 July	20	10	10
(1) GR	27 July	20	10	10
(1) SR	27 July	< 5	< 5	≤ 5
(2) PB	21 Aug.	5	≤ 5	10
(7) EE	26 July	5	10	5
(8/9) BF	7 Sept.	5	≤ 5	≤ 5
(8/9) KF	7 Sept.	< 5	< 5	≤ 5
(11) NT	30 July	5	≤ 5	5
(11) MT	30 July	< 5	< 5	≤ 5
(17) BS	24 July	≤ 5	< 5	< 5
(17) PS	24 July	5	≤ 5	< 5
(17) DS	24 July	20	< 5	≤ 5

supplied milk within the area and also raised sheep and cattle for local slaughter. This farm and several others were visited; manure from cattle, chicken and sheep was found to contain cryptosporidia. Samples of manure spread in fields adjacent to affected households that were used for recreational purposes by some of those affected were also found to contain cryptosporidia.

Milk and milk filter 'socks' failed to yield cryptosporidia, although a milk filter from one farm in the area subsequently visited showed a few objects morphologically indistinguishable from cryptosporidium oocysts. There were, however, too few for confirmation to be substantiated.

A 'country park' recreational development that runs from Holywell to Greenfield consists of a series of interconnected spring-fed ponds associated with industrial archeological sites. This area is used both informally and by school nature-study groups; inspection revealed that effluent from the town abattoir and from both surface and foul domestic drains was gaining access to these ponds. Pond water and several drain swabs taken in the locality yielded cryptosporidia and campylobacters. Children use these ponds for water sports and games. Identification of cryptosporidia from these environmental sources was confirmed by FTA using human positive specific IgA and anti-A conjugate. It was possible to exclude those samples that yielded cryptosporidium-like bodies that failed to fluoresce. It was not, however, possible to assess viability of the oocysts.

Several poultry-producing units were visited and droppings were collected. Most samples yielded campylobacters. One also contained cryptosporidia. Chicken litter is disposed of into fields, often lying in heaps for considerable periods; cattle lie on such heaps.

Serological studies

All *Cryptosporidium*-positive patients from whom blood was obtained showed an antibody response which was primarily in the IgA and IgM classes; control groups showed either no antibody or lower antibody levels, mainly in the IgG class (Table 2). Paired acute and convalescent sera showed significant rising titres. One additional case (case 20) was diagnosed retrospectively by means of a single high antibody titre and gave a history of a characteristic clinical episode which had occurred just before the outbreak was recognized. The patient was a disabled individual who was confined to her home. Two other close family contacts of confirmed cases showed lower levels of antibodies (IgG, A and M), suggesting recent infection but without corroborative clinical information, suggesting that mild or subclinical infections had occurred.

DISCUSSION

There are several unusual features in the outbreak described, including (i) a previously unreported association with campylobacter, (ii) family outbreaks, (iii) few cases had direct contact with livestock or other obvious source of infection. The evidence of person-to-person spread and the lack of evidence of animal contact in many previously reported cases has cast doubt on the origin of such infections (Editorial, 1984; Casemore & Jackson, 1984) and the importance of the infection as a cause of gastrointestinal infection in an urban community has been described (Hunt *et al.* 1984). Vomiting has been a predominant feature in a number of these cases and the presence of *Cryptosporidium* in one sample of vomit is of importance. It may well account, as a result of aspiration, for cases described elsewhere of pulmonary cryptosporidiosis in the immunocompromised and would also provide a potential route of transmission.

In this outbreak a variety of potential sources was present and five possible routes of infection had to be considered:

(1) Contact with animals or their excreta.
(2) Consumption of infected or contaminated milk.
(3) Consumption of contaminated water.
(4) Consumption of infected or contaminated food.
(5) Person-to-person spread.

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The case-control study was initiated early in the outbreak and was designed to test the above possibilities. Although limited by the small number of cases, the evidence obtained, together with subsequent cases and environmental studies, permit certain inferences to be drawn.

The concurrent campylobacter infection suggests a possible common epidemiology in this outbreak. Although, like *Cryptosporidium*, *Campylobacter* sp. is widespread among animals and in the environment, it is often difficult or impossible to determine the source in sporadic cases (Dawkins, Bolton & Hutchinson, 1984). Salmonellosis and campylobacter enteritis from raw or defectively heat-treated milk are thought to be both under-reported and increasing (Galbraith, Forbes & Clifford, 1982; Anonymous, CDSC, 1984). The area studied has an unusually large number of producer/retailers of milk, and both farm-bottled raw and pasteurized milk is supplied to many households in the area. *Cryptosporidium* is known to occur in more than 30% of local herds (DVO, personal communication). Despite the absence of statistical proof from the case-control study, or of confirmed positive findings in the examination of milk, raw milk would appear to provide a possible vehicle for transmission of cryptosporidia and proof may have to await more sensitive detection methods; direct microscopical examination, even with concentration, lacks the sensitivity available for detection of bacteria using enrichment culture. Indeed, one other patient investigated, an 11-year-old male with gastroenteritis, having no connection with this outbreak, yielded both *Cryptosporidium* and campylobacter; he was the only member of his family to consume raw milk.

The case-control study showed a positive association with sausage consumption. Examination for cryptosporidia in meat and meat products is hampered by the constraints described above; nevertheless, sausages would seem to provide a possible vehicle for transmission considering their constituents, mode of manufacture and the possibilities of undercooking; also, inquiry revealed that people often eat pieces of uncooked sausage.

The two families in whom both infections occurred concurrently showed two different patterns of spread. In the first family (cases 8 and 9 and their parents) mixed infection appeared simultaneously, suggesting a common source outbreak. The second family (cases 6, 10, 13) had sequential infections with *Cryptosporidium* but only the first case yielded a campylobacter, which inferred possible person-to-person spread of the *Cryptosporidium*. The index patient suffered from severe vomiting and cachexia. He regularly walked his dog across adjacent farm land from which samples were obtained in which cryptosporidia were found to be present. The demonstration in case 14 of *Cryptosporidium* in vomit is a finding we have not previously seen reported and suggests a possible mechanism of spread of cryptosporidium infection within this family and indeed in other examples of person-to-person transmission. In one case vomiting occurred without obvious diarrhoea while in other cases vomiting preceded the diarrhoea.

Contract disposal to land of both farm and human domestic manure or slurry was carried out by one of the farms investigated. Land used for such disposal, although officially required to be left idle for a period, was subsequently used for grazing and silage cropping for winter feed for their dairy cattle and is also used by residents for recreational purposes. Just before the onset of the outbreak complaints had been received by the Environmental Health Department from local residents of offensive smells arising from 'muck-spreading' which must be considered as a possible route for transmission of cryptosporidium on the evidence obtained. Contamination of water supplies, directly or by such practices, occurs and oocysts are known to be relatively resistant to chlorine (Campbell *et al.* 1982).

Several of the dairy farms raise their own calves. Such calves, when infected may reinfect adjacent dairy cattle, or the stress of transport and lairage of cattle going for slaughter may, by analogy with salmonellae, cause a recrudescence of infection. Both may contribute to the contamination of the environment which, at least in some cases, was probably the direct source of human infection.

Temporal clustering was apparent and the manner in which the outbreak appeared in mid-March and disappeared in mid-May may well be the result of seasonal farming practices together with increased direct human exposure to the environment, which is the subject of continuing study.

In summary, no definite evidence could be adduced, despite intensive investigation, to demonstrate the source of the outbreak but several potential sources were identified and the link must remain circumstantial.

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