

Shedding of oocysts by immunocompetent individuals with cryptosporidiosis

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SUMMARY

Studies were made on 33 immunocompetent patients with cryptosporidiosis (31 of them children) to determine how long oocyst shedding continued after the cessation of diarrhoea. The majority (20/33) ceased to shed oocysts in the week following the cessation of diarrhoea. However 5 patients (15%) continued to shed oocysts for 2 weeks or more, and 1 patient was still shedding oocysts when lost to follow-up 3 weeks after the diarrhoea ceased.

There was considerable variation among patients, and some eliminated oocysts more efficiently and quickly than others. The sucrose flotation technique was more useful than direct smear for detecting the low numbers present at the end of the shedding period. Limited information suggested that a carrier state or relapse is probably rare.

INTRODUCTION

Although human infections caused by the protozoan *Cryptosporidium* were first detected in immunocompromised patients (see e.g. Ma, 1984) evidence is accumulating on the importance of *Cryptosporidium* as a cause of gastroenteritis in immunocompetent individuals (Tzipori *et al.* 1983a; Hunt *et al.* 1984; Hart, Baxby & Blundell, 1984; Holten-Anderson *et al.* 1984).

Interest is being shown in the pattern of oocyst shedding by immunocompetent patients. In a series of 12 adult patients, Current *et al.* (1983) found that oocyst shedding ceased either during, or shortly after, the illness. Hunt *et al.* (1984) detected oocyst shedding in 44% of their 43 cases 2 weeks or more after the onset of symptoms, and called for more information on oocyst excretion. We have previously reported the clinical details of 27 cases and noted that in 15 patients available for follow-up, oocysts continued to be shed for perhaps as long again as the duration of diarrhoea (Hart, Baxby & Blundell, 1984). We now present more information from 33 patients which shows differences in the speed and efficiency with which immunocompetent individuals eliminate oocysts, and the value of the sucrose flotation technique for investigating them.

MATERIALS AND METHODS

Patients

Information was collected on 33 immunocompetent patients (31 of them children). In only one case was another enteropathogen detected (*Escherichia coli* O111). All cases were diagnosed as cryptosporidiosis by direct smear of faeces during the acute phase, and samples of faeces were collected during, and particularly after, the illness. Wherever possible specimens were taken at closely spaced intervals in order to determine precisely when shedding of oocysts ceased.

Direct smears

Oocysts were detected in direct smears of faeces by the safranin-methylene blue staining technique, which stains oocysts more efficiently than modified Ziehl-Neelsen methods (Baxby, Blundell & Hart, 1984). Briefly, after fixation with 3% HCl-methanol, smears were stained with 1% aqueous safranin with vigorous heating for 1 min, and counterstained with 1% aqueous methylene blue.

Sucrose-phenol flotation

Oocysts were concentrated by the sucrose-phenol flotation technique (Current *et al.* 1983) which is probably the most sensitive technique available for the detection of small numbers of oocysts (Kirkpatrick, 1982). The faecal sample was homogenized in 20 ml saline and allowed to stand. The supernatant was decanted and centrifuged at 500 g for 10 min. The pellet was resuspended in 10 ml sucrose-phenol (sucrose 1500 g/l, phenol 19.5 g/l) and centrifuged at 500 g for 10 min. Material from the meniscus was then transferred to a microscope slide, diluted in sucrose-phenol if necessary, and examined under a coverslip by bright-field microscopy (see below).

RESULTS

Detection of oocysts

The safranin-methylene blue technique is very efficient and stains > 90% of oocysts (Baxby, Blundell & Hart, 1984). With experience it is possible to scan the entire area of a stained smear with $\times 20$ objectives and detect just one stained oocyst. If smears are made with a bacteriological loop of volume 1/300 ml this provides a lower limit of sensitivity for the direct smear technique of 3×10^2 oocysts/ml faeces, providing one smear was examined. Direct comparison showed that the sucrose-phenol flotation technique achieved a 20 to 30-fold concentration and would detect *c.* 10 oocysts/ml faeces.

The importance of choice of microscope objectives for the flotation technique has been emphasised (Anderson, 1981; Ma & Soave, 1983). With Kyowa Achromatic 'A' series objectives oocysts were seen as bright pink spherical-ovoid bodies whereas yeasts, a possible cause of confusion, appeared green. Kyowa Planar 'PL' objectives were much less effective. Oocysts are buoyant in sucrose and adhere to the underside of the coverslip above the plane of focus in which yeasts are found (Anderson, 1981). If necessary the top fraction from the flotation can be diluted

Table 1. *Duration of oocyst shedding after cessation of diarrhoea in 33 patients**

Patients	Method	No. (33)	Last positive specimen days \pm s.d. (range)	First negative specimen days \pm s.d. (range)†
33	(A) Smear	11/15	$\geq 4.1 \pm 6.0$ (-3-18)	$\leq 6.3 \pm 5.6$ (-1-19)
	(B) Smear	17/18	$\geq 5.4 \pm 6.1$ (-2-21)	N/A
33	(C) Float	10/11	$\geq 7.0 \pm 6.8$ (1-21)	$\leq 9.2 \pm 6.4$ (3-22)
	(D) Float	22/22	$\geq 7.4 \pm 6.4$ (-1-21)	N/A

* Oocyst shedding timed from cessation of diarrhoea. A negative value indicates that oocyst shedding apparently ceased before the end of diarrhoea. (Duration of diarrhoea 12.1 ± 4.6 days, range 5-25 days.)

† In groups A and C, samples were sufficiently closely spaced to enable an accurate 'first negative' specimen to be obtained. In groups B and D there were longer intervals between the specimens or patients were still shedding oocysts when lost to follow up.

and centrifuged at 500 g for 10 min to allow recovery of the oocysts and confirmation of their identity by staining.

Duration of oocyst shedding

All 33 patients had diarrhoea; the mean duration was 12.1 ± 4.6 days (median, 12 days) with a wide range (5-25 days). The start of oocyst shedding was not known for all patients; with some the first specimen was received relatively late in the illness. Consequently we concentrated on the duration of the oocyst shedding after the cessation of diarrhoea and information on this is shown in Table 1.

When the direct smear technique was used we found that 28/33 patients (85%) excreted oocysts after diarrhoea had ceased (Table 1 A, B). Collection of specimens at closely spaced intervals (≤ 3 days) from 15 patients allowed us to determine when shedding ceased. This was on average between 4 and 6 days after the cessation of diarrhoea. However there was considerable variation among the patients, ranging from apparent cessation of shedding 3 days before the diarrhoea ended to shedding for at least 21 days after.

With the flotation technique oocyst shedding was detected after the cessation of diarrhoea in 32/33 patients and for slightly longer periods (Table 1 C, D). Tests on closely spaced specimens from 11 patients indicated that shedding ceased between 7 and 9 days after the end of diarrhoea (Table 1 C). Again there was considerable variation and the increased duration of shedding detected by sucrose flotation was not significantly longer.

However the general superiority of the flotation technique to determine low numbers for longer periods was generally evident. For example in 4 of the 5 cases which apparently stopped shedding oocysts before the diarrhoea ended sucrose flotation detected oocysts in the specimens recorded as 'first negative' by direct smear. In all 4 cases flotation showed that oocyst shedding continued after cessation of diarrhoea, in 1 case for at least 12 days, and in another for at least 9 days.

In general the duration of oocyst shedding was not related to the duration of diarrhoea (Table 2). The majority of patients shed oocysts for less than 1 week

Table 2. *Duration of oocyst shedding after cessation of diarrhoea*

Duration of shedding (days)*	No. (33)	%	Cumulative		Mean duration of diarrhoea (days)
			No. (33)	%	
<7	19	58	32†	97†	12.9
8-14	8	24	13	39	12.4
>15	5	15	5	15	11.4

* After cessation of diarrhoea.

† Oocysts ceased to be detected in the faeces of 1 patient, 1 day before the diarrhoea ended.

Table 3. *Combined duration of diarrhoea and oocyst shedding*

Duration (days)*	No.	%	Cumulative	
			No.	%
<14	6	18	33	100
15-21	13	40	27	82
22-28	7	21	14	42
>28†	7	21	7	21

* Measured from start of diarrhoea to cessation of oocyst shedding.

† Longest period = 38 days (diarrhoea 17 days).

after the end of diarrhoea. However 13 patients (39%) shed oocysts for more than 1 week and of these, 5 (15%) were still shedding after more than 2 weeks. In fact of these 5 patients 1 shed for 20 days, another for 21 days, and a third was still shedding at 21 days when lost to follow-up. The total probable period of shedding, which may be taken as the duration of diarrhoea plus duration of shedding after diarrhoea ceases, is shown in Table 3. This shows that 27 patients (82%) shed oocysts for more than 2 weeks, 14 (42%) for more than 3 weeks, and 7 (21%) for over 4 weeks. The longest period was in a 2-month-old patient who shed oocysts for 21 days after having diarrhoea for 17 days. Another patient was still excreting large numbers of oocysts when lost to follow up 21 days after a 12-day bout of diarrhoea.

Patterns of oocyst shedding

As described there was considerable variation in the duration of oocyst shedding, and when the patients were considered as a single group the periods detected by flotation were only slightly longer than when detected by smear (Table 1).

However, some patients eliminated oocysts efficiently, i.e. they switched quickly from shedding many oocysts (detectable by direct smear) to undetectable numbers, whereas others eliminated oocysts inefficiently, i.e. they initially shed many oocysts, then fewer for a period (i.e. detectable by flotation but not smear) before the numbers dropped to undetectable levels. Sixteen patients for whom sufficient information was available were ranked by duration of shedding after diarrhoea stopped (Fig. 1). Those who eliminated the parasite efficiently tended to shed for shorter periods after the diarrhoea ceased (patients 1-7, 9, Fig. 1) than those who eliminated the parasite inefficiently (patients 8, 10-16, Fig. 1). If these patients

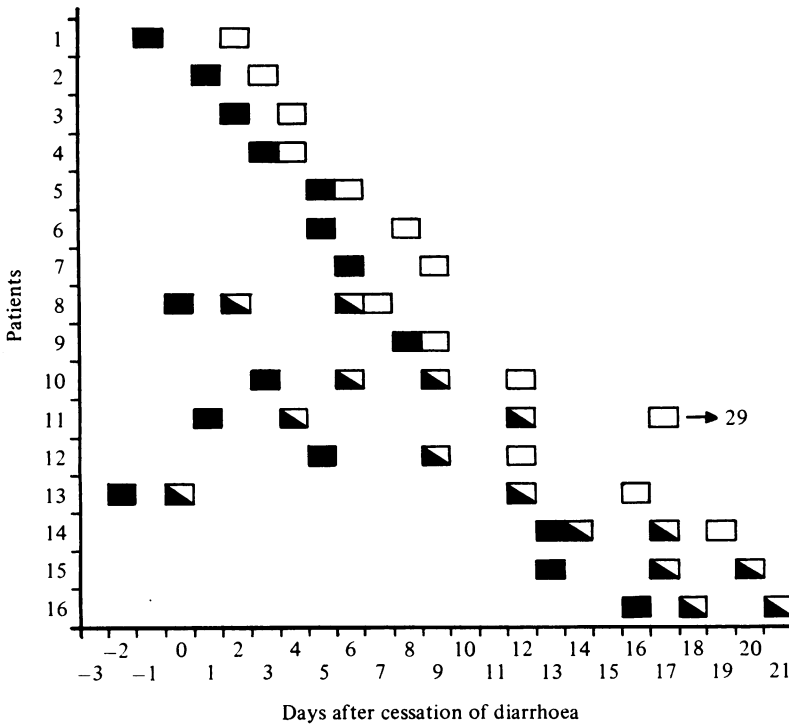


Fig. 1. Shedding of *Cryptosporidium* oocysts by patients, ranked by increasing duration of shedding after cessation of diarrhoea. For each patient is recorded the last occasion on which oocysts were detected by both direct smear and sucrose flotation (■); the first and last days on which oocysts were detected by flotation but not by direct smear (◑); and, where possible, the first day when oocysts were not detected by either method (□). For clarity earlier smear positive and intermediate smear negative float positive findings are omitted.

are separated into two groups then the duration of shedding by the 'inefficient' patients (13.3 ± 6.7 days) was significantly longer ($P < 0.001$) than that by the efficient patients (3.6 ± 2.9 days). All patients may in fact shed low numbers for a time before eventually eliminating the parasite. However in the efficient group this period must be short, generally less than 2 days and in some cases (patients 4, 5, 9) less than 1 day. On the other hand the shortest period of low-level shedding by inefficient patients was at least 4 days.

Long-term follow-up

Usually no more specimens were received from a patient after sucrose flotation failed to detect oocysts in two or three specimens taken during a 7-day period. One patient presented with diarrhoea again 6 months after recovery, and cryptosporidiosis was again diagnosed. Unfortunately we could not tell whether this represented relapse or reinfection. However occasional specimens continued to be received from other patients, in one case for up to 13 months. No further evidence of oocyst shedding by these patients was detected once the initial phase of shedding had ceased, even in four children who subsequently had other gastro-intestinal infections. This suggests that immunocompetent patients eliminate *Cryptospor-*

idium effectively, and that a carrier state leading to relapse or to reactivation by other enteropathogens may be rare.

DISCUSSION

So far relatively little information has been collected on the duration of oocyst shedding in human cryptosporidiosis. In infected lambs and calves shedding coincides with diarrhoea or ceases shortly after (Anderson, 1981, 1982; Tzipori *et al.* 1983*b*). Although recognition of oocysts in suitably stained direct smears of faeces is a convenient method for laboratory diagnosis of cryptosporidiosis (Baxby, Blundell & Hart, 1984), this paper emphasises the value of the sucrose-phenol flotation technique for monitoring the shedding of oocysts, particularly after diarrhoea ceases. In a series of 12 human patients Current *et al.* (1983), who used sucrose flotation, found that oocyst shedding ceased during the clinical phase or shortly after. Hunt *et al.* (1984), who used a modified Ziehl-Neelsen stain, noted that 44% of their 43 cases shed oocysts for 2 weeks or more after the onset of illness. In our series using sucrose flotation we found that 27/33 patients (82%) shed oocysts for more than 2 weeks, 14 patients (42%) for more than 3 weeks and 7 patients (21%) for more than 4 weeks from the onset of symptoms.

When results obtained with sucrose flotation and direct smear techniques were compared, differences were found in the efficiency with which oocysts were eliminated. Sufficiently detailed information was available from only 16 patients but it appears that efficient elimination is associated with short periods of shedding after diarrhoea and inefficient elimination with long periods. It is thus possible that two patterns of shedding may occur although more information is needed to test this hypothesis. None of the features examined namely age, sex, severity of diarrhoea or other symptoms correlated with these possible patterns except duration of diarrhoea, which was slightly longer (16 days) in the efficient group than in the other (12 days).

Although initially considered as a zoonosis (Schultz, 1983) evidence is accumulating that cryptosporidiosis may be transmitted from person-to-person (Baxby, Hart & Taylor, 1983; Collier, Miller & Meyers, 1984; Koch *et al.* 1985). Oocyst shedding during the period of diarrhoea is to be expected and appropriate precautions can be taken. Consequently from an epidemiological viewpoint, oocyst shedding after the cessation of diarrhoea may be more important. The majority of our patients (60%) shed oocysts for less than 1 week after the cessation of diarrhoea and this contributed to the low average shedding time. However five patients (15%) were still shedding oocysts more than 2 weeks after the end of diarrhoea (Table 2). The longest total period was in a 2-month-old patient who shed oocysts for 21 days after having diarrhoea for 17 days. Another patient was still excreting large numbers of oocysts when lost to follow-up 21 days after a 12-day period of diarrhoea.

Although sucrose flotation is a sensitive technique (Kirkpatrick, 1982) it is possible that occasional oocysts may be present in very low numbers in some samples and not detected. The infective dose of *Cryptosporidium* is unknown and oocysts are resistant to many disinfectants (Campbell *et al.* 1982). Consequently it would be reasonable to regard patients as infective until oocyst shedding ceases,

and patients in hospital should be nursed in isolation. Parents of patients should be counselled on basic hygiene and on modes of spread, particularly if young children are involved. What little evidence is available suggests that a carrier state or relapse may be rare.

REFERENCES

- ANDERSON, B. C. (1981). Patterns of shedding of cryptosporidial oocysts in Idaho calves. *Journal of the American Veterinary Medical Association* **178**, 982-984.
- ANDERSON, B. C. (1982). Cryptosporidiosis in Idaho lambs: natural and experimental infections. *Journal of the American Veterinary Medical Association* **181**, 151-153.
- BAXBY, D., BLUNDELL, N. & HART, C. A. (1984). The development and performance of a simple, sensitive method for the detection of *Cryptosporidium* oocysts in faeces. *Journal of Hygiene* **92**, 317-323.
- BAXBY, D., HART, C. A. & TAYLOR, C. (1983). Human cryptosporidiosis: a possible case of hospital cross-infection. *British Medical Journal* **287**, 1760-1761.
- CAMPBELL, I., TZIPORI, S., HUTCHINSON, G. & ANGUS, K. W. (1982). The effect of disinfectants on the survival of *Cryptosporidium* oocysts. *Veterinary Record* **111**, 414-415.
- COLLIER, A. C., MILLER, R. A., & MEYERS, J. D. (1984). Cryptosporidiosis after marrow transplantation: person-to-person transmission and treatment with spiramycin. *Annals of Internal Medicine* **101**, 205-206.
- CURRENT, W. L., REESE, N. C., ERNST, J. V., BAILEY, W. S., HEYMAN, M. B. & WEINSTEIN, W. M. (1983). Human cryptosporidiosis in immunocompetent and immunodeficient persons. *New England Journal of Medicine* **308**, 1252-1257.
- HART, C. A., BAXBY, D. & BLUNDELL, N. (1984). Gastro-enteritis due to *Cryptosporidium*: a prospective survey in a children's hospital. *Journal of Infection* **9**, 264-270.
- HOLTEN-ANDERSON, W., GERSTOFT, J., HENRIKSEN, Sv. Aa & PEDERSEN, N. S. (1984). Prevalence of *Cryptosporidium* among patients with acute enteric infection. *Journal of Infection* **9**, 277-282.
- HUNT, D. A., SHANNON, R., PALMER, S. R. & JEPHCOTT, A. E. (1984). Cryptosporidiosis in an urban community. *British Medical Journal* **298**, 814-816.
- KIRKPATRICK, C. E. (1982). Techniques for the diagnosis of intestinal parasites. *Animal Health Techniques* **3**, 324-331.
- KOCH, K. L., PHILLIPS, D. J., ABER, R. C. & CURRENT, W. L. (1985). Cryptosporidiosis in hospital personnel; evidence for person-to-person transmission. *Annals of Internal Medicine* **102**, 593-596.
- MA, P. (1984). *Cryptosporidium* and the enteropathy of immune deficiency. *Journal of Pediatric Gastroenterology and Nutrition* **3**, 488-490.
- MA, P. & SOAVE, R. (1983). Three-step stool examination for cryptosporidiosis in 10 homosexual men with protracted watery diarrhoea. *Journal of Infectious Diseases* **147**, 824-828.
- SCHULTZ, M. G. (1983). Emerging zoonoses. *New England Journal of Medicine* **308**, 1285-1286.
- TZIPORI, S., SMITH, M., BIRCH, C., BARNES, G. & BISHOP, R. (1983a). Cryptosporidiosis in hospital patients with gastroenteritis. *American Journal of Tropical Medicine and Hygiene* **32**, 931-934.
- TZIPORI, S., SMITH, M., HALPIN, C., ANGUS, K. W., SHERWOOD, D. & CAMPBELL, I. (1983b). Experimental cryptosporidiosis in calves: clinical manifestations and pathological findings. *Veterinary Record* **112**, 116-120.