

## CRYPTOSPORIDIUM CARRIAGE IN ASYMPOTOMATIC RURAL SOUTH INDIANS

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### ABSTRACT

A sudden increase in *Cryptosporidium* oocyst excretion in a community under surveillance for intestinal parasite carriage was investigated. Intermittent oocyst excretion was seen in 11 individuals who remained asymptomatic over a period of four months. *Cryptosporidium* oocysts were found in one water source and in buffalo dung. While oocyst excretion by asymptomatic individuals has been reported earlier, oocyst passers have not been followed up to determine whether they remain asymptomatic or develop diarrhoea. This is the first documented confirmation of repeated excretion over a prolonged period, indicating asymptomatic colonization by *Cryptosporidium*.

KEY WORDS : Asymptomatic carriage, *Cryptosporidium*

### Introduction

The protozoan parasite *Cryptosporidium* sp is known to be a common cause of diarrhoea in a variety of settings.<sup>1</sup> In an earlier report from Vellore,<sup>2</sup> we had reported virtually identical *Cryptosporidium* excretion rates in patients with diarrhoea and in a comparable group of healthy asymptomatic subjects. The most common mode of transmission of this coccidian parasite is considered to be faeco-oral from infected humans and animals. Contamination of water sources is also of importance.<sup>3</sup> Cases may be sporadic, but small clusters, family and large community-wide outbreaks also occur. While presence of *Cryptosporidium* oocysts in stool indicates infection, the absence of oocysts on a single faecal examination does not rule out infection since oocyst excretion may be intermittent. While studies have previously shown that asymptomatic oocyst excretion rates of 1.5% in controls<sup>4</sup> and 12.7% in immunocompetent patients undergoing upper gastrointestinal endoscopy,<sup>5</sup> none of these studies followed up the oocyst passers to determine whether they subsequently developed diarrhoea.

This report documents asymptomatic carriage of *Cryptosporidium* for over 4 months in 11 subjects of whom 9 were previously unaffected.

### Material and Methods

The residents of a village 35 km from Vellore were under surveillance for bacterial and parasitic enteric pathogens for the period January 1994 to January 1996. Stools were collected from a stratified random sample of 20% of the village of 1400 people, 20-30 of whom provided samples once a week. Each individual was sampled at intervals of approximately 3 months. From January 1994 to October 1994, 1087 faecal specimens were collected and of these 52 (4.78%, usually 1-2 samples per week) were

positive for *Cryptosporidium* oocysts. In the first week of November 1994, 11 individuals were found to excrete *Cryptosporidium* oocysts and it was decided to follow them to see whether they continued to excrete *Cryptosporidium* oocysts asymptotically, or developed diarrhoea. Subsequently stool samples were collected from them every two weeks for four months. Stool samples were also collected from the livestock belonging to these individuals. These consisted of two cows and one buffalo.

### Examination of water sources

A total of four water sources were used by the 11 subjects. These were one hand pump, one bore well and two open wells. Five litres of water was collected from all water sources used by the index families and transported immediately to the laboratory for processing within 2 hours of collection. The water sample was filtered through a 3 micron membrane filter, backwashed with 50 ml of distilled water, centrifuged at 10,000 rpm for 30 minutes and the deposit examined for *Cryptosporidium* oocysts, by the safranine-methylene blue and the fluorescent monoclonal antibody techniques as described below.

### Processing of stool samples

Stool samples from humans and freshly passed samples of dung from livestock were transported on ice to the laboratory, concentrated by the formol-ether technique and 2 slides made from each sample for staining with safranine-methylene blue as previously reported.<sup>2</sup> Another smear was prepared from all samples which had typical orange-red acid-fast oocysts measuring 4-6 microns in size<sup>6</sup> and the identification confirmed by staining with fluorescein-isothiocyanate tagged monoclonal antibody directed against the oocyst wall (a gift from Mr. A.H. Moody, Hospital for Tropical Diseases, London).

### Results

The background rate of *Cryptosporidium* oocyst excretion was 4.78% until October 1994, similar to the rates documented earlier.<sup>2</sup> In the second week of November

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Received : 14-03-1998.

Revised : 02-05-1998.

Accepted : 19-06-1998.

1994, there was a sudden increase with 33% (11/33) of samples received from the village being positive for *Cryptosporidium* oocysts.

Stool samples from all 11 index individuals had been examined at least once during the preceding surveillance period of 10 months and 9 were negative. A 4-year-old girl and her 25-year-old mother had oocysts on one of two previous stool examinations. There were 9 females and 2 males, ranging in age from 4 to 45 years.

Two families owned a cow each and 1 family owned a buffalo. A total of 4 water sources used by these families were identified. An open well used by 3 of the families and the dung of the buffalo contained *Cryptosporidium* oocysts in the samples when examined in November but all subsequent samples were negative for oocysts.

Samples were collected from all available subjects for a total of 7 times from 15/11/1994 to 2/3/1995 (Table). Since intermittent oocyst excretion continued over this period in all 11 subjects and none developed any gastrointestinal symptoms no further follow up was done. During this period in all individuals at least 2 samples did not contain oocysts. On 2/3/1995, 8 of the 11 individuals were excreting oocysts. The continuing routine surveillance of the other members of the community showed a rate of *Cryptosporidium* infection ranging from 5 to 10%.

Subjects after a period of 4 months indicates that infection could have been acquired following an acute infective episode, simultaneously from one or more sources, possibly the open well and the buffalo and this resulted in an asymptomatic colonization. While contaminated water and animal dung were identified as potential sources of infection these could be correlated only with 8 of 11 subjects. However, for 3 subjects the potential source of infection could not be identified. It is possible that other water sources used by these individuals were also contaminated but this was not detected by our examination.

Oocyst excretion is intermittent and in an environment that is contaminated the potential sources of infection are many. The findings reported here document asymptomatic *Cryptosporidium* carriage, probably following colonisation with frequent but intermittent oocyst excretion. Although it is possible that these individuals were not colonised, but were infected repeatedly over the four month period, it is unlikely because no oocysts were found after November 1994 in the water or dung samples, which were the potential sources of infection. Additionally, the rest of the cohort under surveillance continued to have oocyst excretion levels of about 5%. In societies in developing countries, with poor environmental hygiene, the mechanisms by which this intracellular but extracytoplasmic parasite can colonise the

Table. Oocyst excretion by 11 asymptomatic individuals from November 1994 to March 1995.

| S. No. | Age /Sex | House No. | Oocyst excretion |      |       |      |      |     |     |
|--------|----------|-----------|------------------|------|-------|------|------|-----|-----|
|        |          |           | 15/11            | 7/12 | 21/12 | 11/1 | 25/1 | 8/2 | 2/3 |
| KM757* | 4/F      | H3/42     | +                | -    | +     | -    | -    | +   | +   |
| KM758* | 25/F     | H3/42     | +                | +    | -     | +    | -    | -   | +   |
| KM759  | 19/F     | H72/4     | +                | -    | NA    | -    | +    | -   | -   |
| KM761  | 16/F     | H78/7     | +                | +    | NA    | +    | -    | NA  | +   |
| KM766  | 37/M     | K188      | +                | NA   | -     | NA   | +    | -   | +   |
| KM768  | 24/F     | K188      | +                | NA   | -     | +    | +    | -   | +   |
| KM769  | 9/M      | K188      | +                | +    | -     | NA   | -    | +   | +   |
| KM774  | 25/F     | K182      | +                | -    | +     | -    | +    | NA  | +   |
| KM775  | 6/F      | K182      | +                | +    | +     | +    | -    | +   | -   |
| KM777  | 45/F     | K181      | +                | -    | -     | +    | -    | +   | -   |
| KM778  | 15/F     | K181      | +                | +    | -     | -    | -    | -   | +   |

\* - previously positive for *Cryptosporidium*      NA - sample not available

#### Discussion

The clustering of *Cryptosporidium* in 11 individuals in 6 families may have been a random finding in a population with upto 10% prevalence of the parasite during the period of surveillance but the continued excretion of oocysts by 8

lining of the gut, grow and be excreted in numbers large enough to be detected on stool examination, while the chronically colonised individual remains asymptomatic are not yet understood.

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