

## SHORT REPORT: POSSIBLE *CRYPTOSPORIDIUM MURIS* INFECTION IN HUMANS

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**Abstract.** Oocysts of cryptosporidia whose morphology resembled that of *Cryptosporidium muris* were found in the stool of 2 healthy girls in Surabaya, Indonesia. The oocysts were predominantly oval and measured  $7.75 \pm 0.17 \times 5.55 \pm 0.13 \mu\text{m}$  (mean  $\pm$  SD). The number of oocysts excreted were more than  $10^5$  per gram of stool. The oocysts were well stained with fluorescein-conjugated monoclonal antibody to *Cryptosporidium*. The specimens from both girls containing the oocysts showed a positive result by the polymerase chain reaction (PCR) using primers specific for the genus *Cryptosporidium*, but a negative result by the PCR using primers specific for *C. parvum*. The 2 girls passed oocysts for 5 and 6 days, respectively. They did not complain of any symptoms during the passage of oocysts.

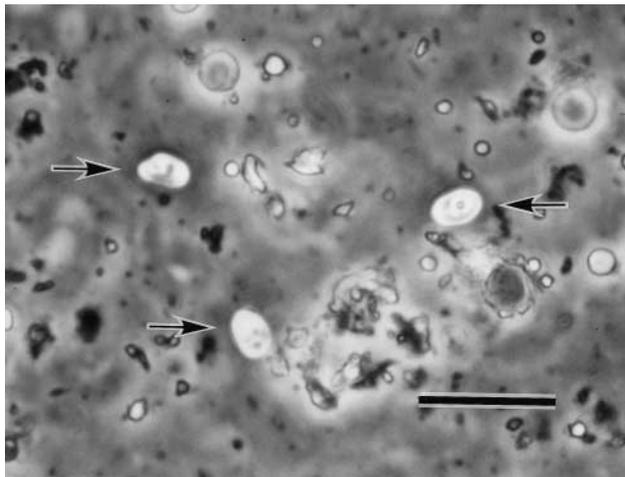


FIGURE 1. Phase-contrast microscopy of *Cryptosporidium* oocysts (arrows) from a healthy girl. The oocysts appear bright and birefringent, contain black residual bodies, are oval, and measure  $7.75 \pm 0.17 \times 5.55 \pm 0.13 \mu\text{m}$  (mean  $\pm$  SD). Bar =  $20 \mu\text{m}$ .

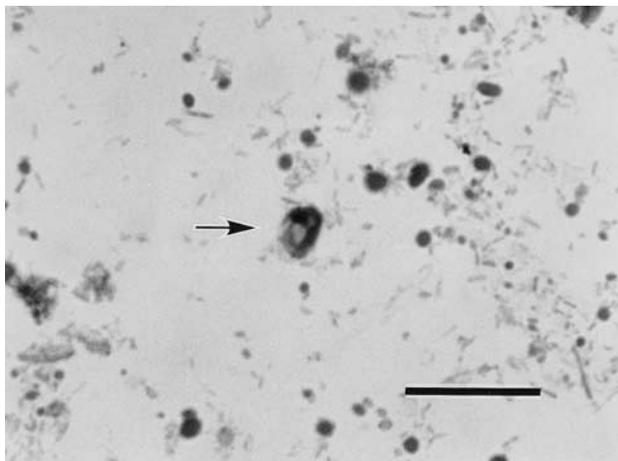


FIGURE 2. Bright-field microscopy of a *Cryptosporidium* oocyst (arrow) from a healthy girl. The oocyst contains sporozoites and a residue of numerous small granules (Kinyoun-modified acid-fast stain, bar =  $20 \mu\text{m}$ ).

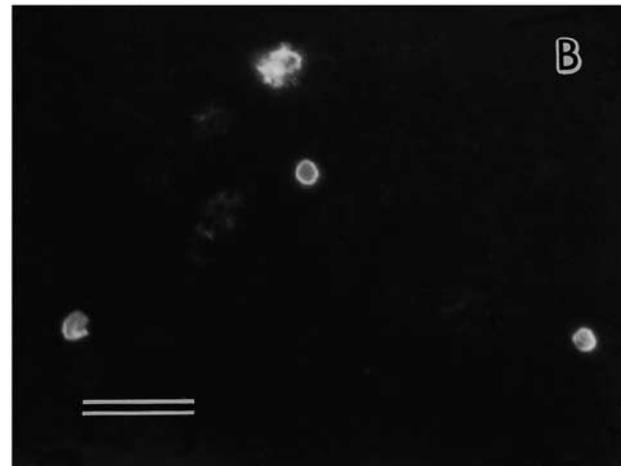
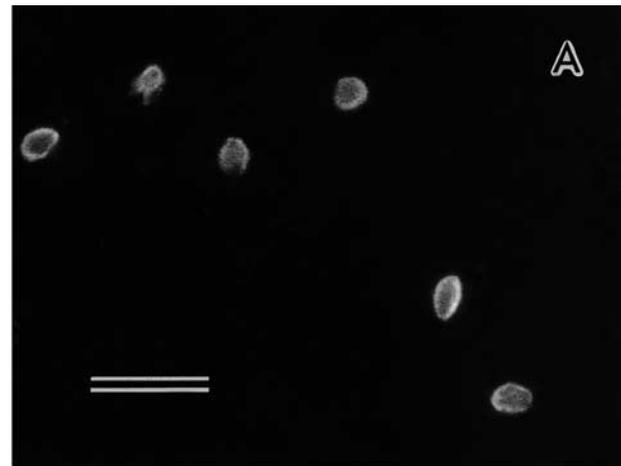


FIGURE 3. *Cryptosporidium* oocysts stained by a direct immunofluorescent antibody procedure. **A**, large oocysts from a healthy girl. **B**, *C. parvum* oocysts from a patient with diarrhea in Indonesia. Bars =  $20 \mu\text{m}$ .

Cryptosporidiosis is an emerging disease that poses a serious threat to the people throughout the world. The gravity of cryptosporidiosis in patients with acquired immunodeficiency syndrome and the outbreak of this disease in communities have been reviewed.<sup>1</sup> Asymptomatic infections have also been detected with increased frequency in many

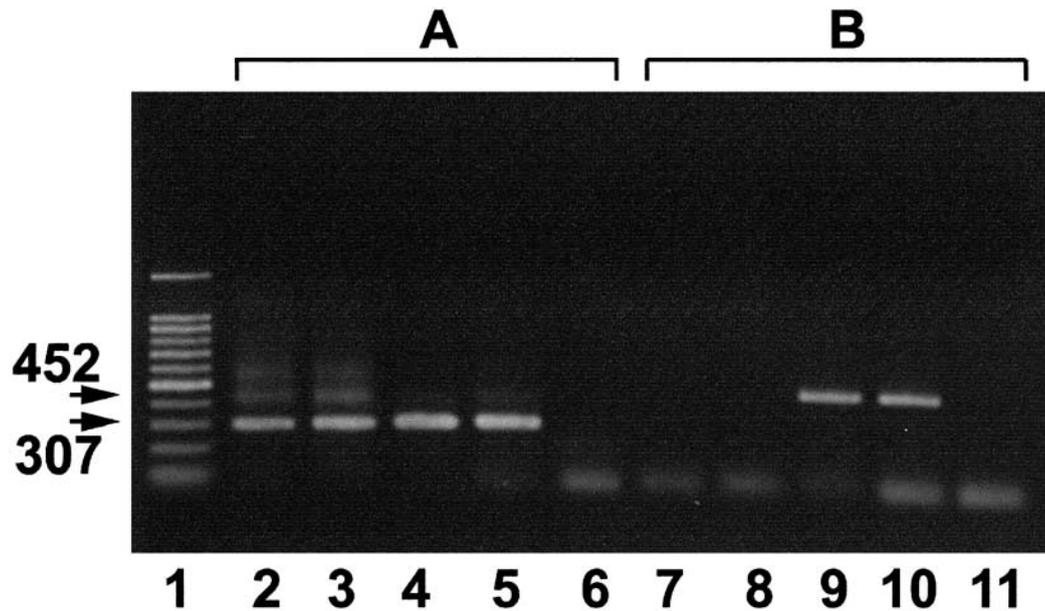


FIGURE 4. Agarose gel electrophoresis of polymerase chain reaction (PCR) products stained with ethidium bromide. Lane 1, 100-basepair DNA ladder (Takara Shuzo Co., Ltd., Osu, Japan). **A**, PCR products (307 basepairs) obtained with primers specific for the genus *Cryptosporidium*. Lane 2, *C. muris* DNA (bovine origin from Japan); lane 3, *Cryptosporidium* DNA from a healthy girl in Indonesia; lane 4, *C. parvum* DNA (human origin from Japan); lane 5, *C. parvum* DNA (human origin from Indonesia); lane 6, negative control. **B**, PCR products (452 basepairs) obtained with primers specific for *C. parvum*. Lane 7, *C. muris* DNA (bovine origin from Japan); lane 8, *Cryptosporidium* DNA from a healthy girl in Indonesia; lane 9, *C. parvum* DNA (human origin from Japan); lane 10, *C. parvum* DNA (human origin from Indonesia); lane 11, negative control. Values on the left are in basepairs.

healthy individuals.<sup>2-5</sup> Since cryptosporidiosis is a zoonotic disease,<sup>6</sup> the source of human cryptosporidiosis could be some species of parasite that infect mammals, birds, and other animals.<sup>3,7,8</sup> However, the parasites isolated so far from patients with diarrhea and healthy individuals are exclusively *C. parvum*.

The present study deals with 2 instances of presumed asymptomatic infection with *C. muris* in Indonesia. We carried out an epidemiologic survey on *Cryptosporidium* infection in Surabaya, Indonesia from August 1992 to July 1993. Stool specimens of patients with diarrhea (1,174 samples) and healthy individuals (5,154 samples) were examined for oocysts of *Cryptosporidium*. Each stool sample was first subjected to Sheather's sugar flotation and smeared on a slide. The slides were examined by phase-contrast microscopy at 600 $\times$  magnification. Once oocysts appearing as phase-bright, birefringent bodies containing black residual bodies against a dark background were identified, the remaining concentrated sample was overlaid with saline and stained using the Kinyoun-modified acid-fast technique. The identification of *Cryptosporidium* was done using taxonomic characteristics, a direct monoclonal antibody immunofluorescent staining method (Crypto-Cel; Cellabs, Brookvale, Australia), which reacts with oocysts of both *C. parvum* and *C. muris*, and the polymerase chain reaction (PCR) with primers specific for the genus *Cryptosporidium* and the primers specific for *C. parvum*.<sup>9-11</sup> To estimate the oocysts concentration in stool, it was weighed and suspended in water. An aliquot of stool suspension was examined for oocysts by using the Kinyoun-modified acid-fast technique.<sup>12</sup> The number of oocysts per gram of stool was then calculated from the mean number of oocysts in 3 samples.

Informed consent was obtained from the parents of both girls (see below). The study was approved by the Ethical Committee of the Tropical Disease Research Center of Airlangga University.

The prevalence of *C. parvum* infection has been reported elsewhere.<sup>13</sup> In addition to subjects in a community who passed oocysts of *C. parvum*, 2 healthy girls (4 and 5 years old) passed oocysts that were morphologically different from those of *C. parvum*. The oocysts were predominantly oval and measured  $7.73 \pm 0.17 \times 5.54 \pm 0.12 \mu\text{m}$  (mean  $\pm$  SD) in 1 case and  $7.77 \pm 0.16 \times 5.57 \pm 0.14 \mu\text{m}$  in the other. These measurements were equivalent to oocyst dimensions published for the bovine *C. muris* oocysts (mean length =  $7.4 \mu\text{m}$ , range =  $6.6-7.9 \mu\text{m}$ , mean width =  $5.6 \mu\text{m}$ , range =  $5.3-6.5 \mu\text{m}$ ).<sup>14</sup> They were significantly larger than oocysts of *C. parvum* ( $5.0 \times 4.5 \mu\text{m}$ ) and contained sporozoites and a residue consisting of numerous, small granules (Figures 1 and 2).<sup>3</sup> They were stained with monoclonal antibody specific for oocysts of *Cryptosporidium* (Figure 3). When we tested the oocysts by PCR with primers specific for the genus *Cryptosporidium*, both the specimen containing possible *C. muris* oocysts and the specimen containing *C. parvum* oocysts were positive. However, when we tested the oocysts by PCR with primers specific for *C. parvum*, the specimen containing *C. parvum* oocysts was positive, but the specimen containing possible *C. muris* oocysts was negative (Figure 4). The number of oocysts excreted at the first observation was more than  $10^5/\text{gram}$  of stool. The oocysts were found in stool of 1 girl for 5 days and in the other girl for 6 days. The girls did not complain of any symptoms during the passage of the large oocysts and did not pass *C. parvum* oocysts.

The oocysts we found in specimens from Surabaya, In-

Indonesia closely resembled the oocysts of *C. muris* in terms of the size and morphologic characteristics. These oocysts were well stained by a monoclonal antibody specific for oocysts of both *C. parvum* and *C. muris*. The results of the PCR showed that the oocysts belonged to the genus *Cryptosporidium*, but were not *C. parvum*. Unfortunately due to the limited amount of field material and infrastructure for animal experiments, the inoculation of oocysts into experimental animals could not be done to confirm the species of the parasite excreted by the 2 girls. However, this study indicates that *Cryptosporidium* oocysts different from those of *C. parvum* were excreted in human stool. The present study strongly suggests that infection with *C. muris* occurs in humans.

**Acknowledgments:** We are indebted to Dr. Yoshiki Aoki, Dr. Hiroji Kanbara (University of Nagasaki, Nagasaki, Japan), and Dr. Motohiro Iseki (Osaka Municipal University, Osaka, Japan) for providing information and advice.

**Financial support:** This study was supported by the Japan International Cooperation Agency.

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