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<td>Author(s)</td>
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SHORT REPORT: POSSIBLE CRYPTOSPORIDIUM MURIS INFECTION IN HUMANS

TATSUYA KATSUMATA, DANIEL HOSEA, IGN GDE RANUH, SHOJI UGA, TETSUO YANAGI, AND SHIGERU KOHNO

Department of Parasitology and Department of Protozoology, Institute of Tropical Medicine, Nagasaki University, Nagasaki, Japan; Tropical Disease Research Center, Airlangga University, Surabaya, Indonesia; Department of Medical Technology, Faculty of Health Science, Kobe University School of Medicine, Kobe, Japan; Second Department of Internal Medicine, Nagasaki University School of Medicine, Nagasaki, Japan

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 ± 0.17 × 5.55 ± 0.13 μm (mean ± SD). The number of oocysts excreted were more than 10⁵ 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.
The slides were examined by phase-contrast microscopy at 400× 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.4-11 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.12 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.13 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 ± 0.17 × 5.54 ± 0.12 μm (mean ± SD) in 1 case and 7.77 ± 0.16 × 5.57 ± 0.14 μm in the other. These measurements were equivalent to oocyst dimensions published for the bovine C. muris oocysts (mean length = 7.4 μm, range = 6.6–7.9 μm, mean width = 5.6 μm, range = 5.3–6.5 μm).14 They were significantly larger than oocysts of C. parvum (5.0 × 4.5 μm) and contained sporozoites and a residue consisting of numerous, small granules (Figures 1 and 2).3 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 10⁴/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, 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 Cryptosporidium. The prevalence of infection has been reported elsewhere.13 Since cryptosporidiosis is a zoonotic disease,6 the source of human cryptosporidiosis could be some species of parasite that infect mammals, birds, and other animals.3,7,8 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× 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.4-11 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.12 The number of oocysts per gram of stool was then calculated from the mean number of oocysts in 3 samples.

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, Indonesia.
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.

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Authors’ addresses: Tatsuya Katsumata, Department of Parasitology, Institute of Tropical Medicine, Nagasaki University, 1-12-4 Sakamoto, Nagasaki 852-8523, Japan. Daniel Hosea and Ign Gde Ranuh, Tropical Disease Research Center, Airlangga University, JL, Dharmahusada 47, Surabaya, Indonesia. Shoji Uga, Department of Medical Technology, Faculty of Health Science, Kobe University School of Medicine, 7-10-2 Tomogakoe, Sumaku, Kobe 654-0142, Japan. Tetsuo Yanagi, Department of Protozoology, Institute of Tropical Medicine, Nagasaki University, 1-12-4 Sakamoto, Nagasaki 852-8523, Japan. Shigeru Kohno, Second Department of Internal Medicine, Nagasaki University School of Medicine, 1-7-1 Sakamoto, Nagasaki 852-8523, Japan.

Reprint requests: Tatsuya Katsumata, Department of Parasitology, Institute of Tropical Medicine, Nagasaki University, 1-12-4 Sakamoto, Nagasaki 852-8523, Japan.

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