

## A Case of Human Cyclosporiasis Causing Traveler's Diarrhea after Visiting Indonesia

This is the first case of human cyclosporiasis reported in Korea. We detected the oocyst of *Cyclospora cayetanensis* from a 14-yr old girl who complained of persistent diarrhea after traveling to Indonesia. Round oocysts sized about 8 to 9  $\mu\text{m}$  with wrinkle on the wall were found in modified acid fast stained stool specimen. Stainability was variable from red to pale. Oocyst wall showed typical autofluorescence under ultraviolet illumination. The exact diagnosis for the cause of diarrhea and treatment for this patient were not provided at the right moment from the hospital since the diagnostic system for the *Cyclospora* infection was not ready in the clinical laboratory of the hospital. More attention should be paid on *Cyclospora* as a cause of diarrhea especially for those returning from a trip to the tropics and an adequate diagnostic system for the *Cyclospora* infection should be implemented in clinical laboratories as soon as possible.

Key Words : *Cyclospora*; Travel; Diarrhea; Indonesia

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### INTRODUCTION

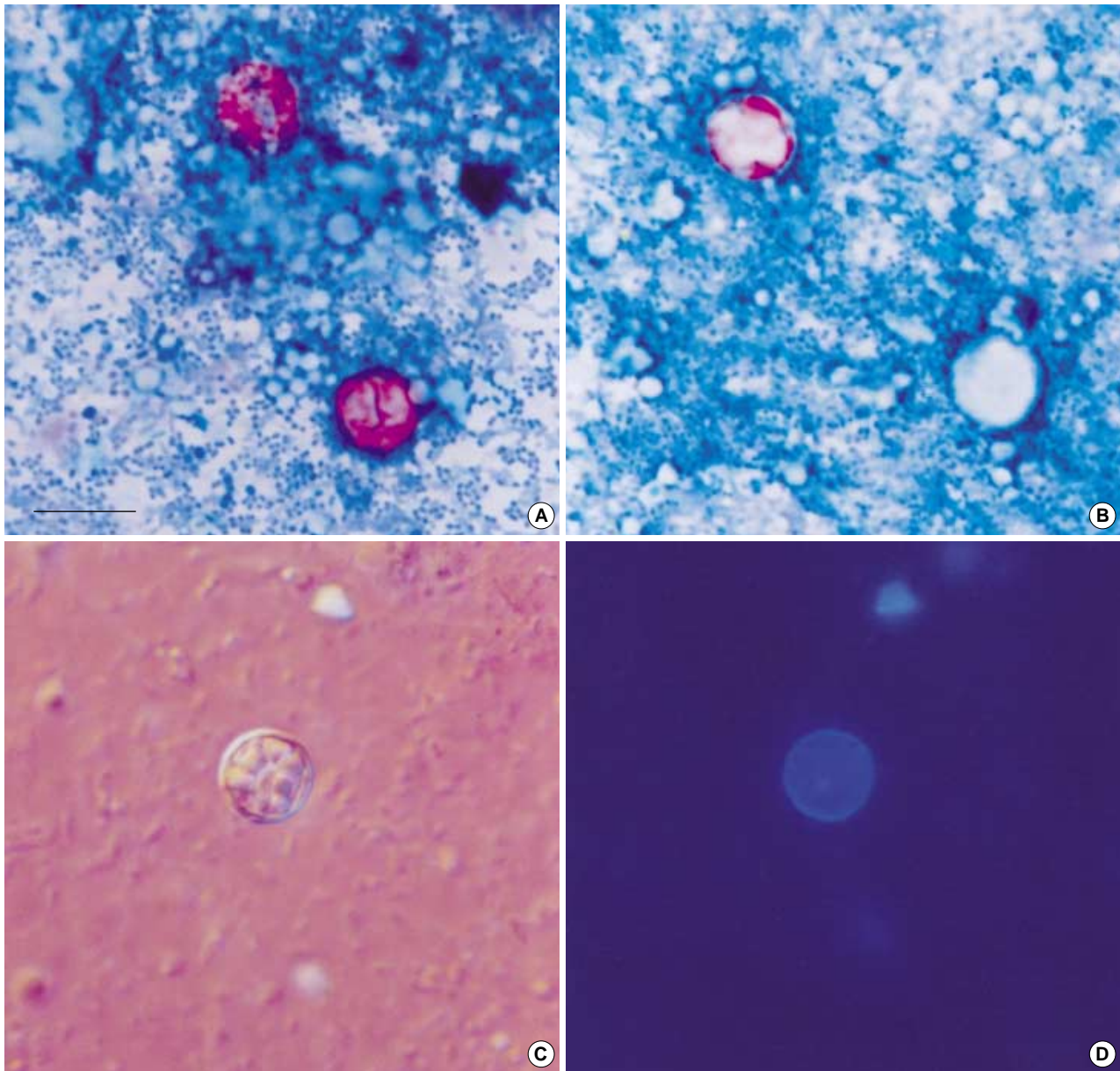
*Cyclospora cayetanensis* is a coccidian protozoa newly recognized as being associated with diarrheal illness. This organism causes traveler's diarrhea among travelers in some developing countries, and especially in west Java, Indonesia, *C. cayetanensis* and *Giardia lamblia* are known as main agents commonly associated with gastroenteritis to Western expatriates (1, 2). We report here a case of cyclosporiasis provoking diarrheal illness during traveling to Indonesia.

### CASE REPORT

A 14-yr old female patient visited the Department of Family Medicine of Gyeongsang National University Hospital located in Jinju-si, Gyeongsangnam-do on December 21, 2001. Her chief complaint was a diarrhea which was watery and had persisted for five days with 5-6 times a day. Other symptoms were not remarkable except for nausea. In her past history, she had visited Bali and Jakarta, Indonesia with her family for 16 days from December 5 to December 20, and diarrhea began from December 16 when she was still in Indonesia. Laboratory tests including Widal test, nested PCR for *Salmonella typhi*, blood culture, stool culture, and parasite examination done by clinical laboratory in the hospital showed all nega-

tive results. Only positive findings were the increased monocyte number (9.9%; normal 3-8%) and erythrocyte sedimentation rate (38 mm/hr; normal 0-20 mm/hr) in complete blood count. The patient was prescribed some medicine on irritable bowel syndrome, but diarrhea was not improved and continued for 15 days more and self limited without any specific treatment. Among three other family members who accompanied her, father showed similar symptoms, and admitted at the local hospital in Jakarta.

The rest of the patient's stool was referred to the laboratory of Department of Parasitology, College of Medicine, Konkuk University for coccidian protozoa, e.g., *Cryptosporidium* and *Cyclospora*. All the stools were smeared on the slide glasses and stained by modified acid fast method after formalin ether sedimentation. Wet smeared sample was prepared and observed under ultraviolet (UV) illumination using epifluorescent microscope with excitation filter BP330-385 (Olympus Optical Co., Japan) if needed. Parasite DNA was extracted from the feces using QIAamp<sup>®</sup> stool mini kit (QIAGEN Inc., CA) and nested PCR against 18S rRNA gene of *Cyclospora cayetanensis* was done with the primers reported by Relman et al. (3). PCR amplification was performed in 50  $\mu\text{L}$  volumes containing 20  $\mu\text{L}$  of template DNA, 10 mM Tris-HCl (pH 9.0), 50 mM KCl, 0.1% Triton X-100, 2 mM MgCl<sub>2</sub>, 200  $\mu\text{M}$  each dATP, dCTP, dGTP, and dTTP, 0.2  $\mu\text{M}$  each primers FIE (5' -TACCCAATGAAAACAGTTT-3') and R2B (5' -CAG-



**Fig. 1.** Oocyst of *Cyclospora cayetanensis* detected from the patient's stool ( $\times 1,000$ ). (A) Round red colored body with wrinkle on the wall on modified acid fast staining. (B) Oocysts showing variable stainability from pale to pink on modified acid fast staining. (C) Oocyst observed by Normarski Differential Interference Contrast microscope. (D) Same oocyst as in C showing autofluorescence along the oocyst wall under UV illumination with excitation filter BP330-385, Olympus. Bar, 10  $\mu\text{m}$ .

GAGAAGCCAAGGTAGG-3') and 1.25 units of *Taq* DNA polymerase. The cycling program consisted of 35 cycles of denaturation at 94°C for 30 sec, annealing at 53°C for 30 sec, and extension at 72°C for 1 min 30 sec. A final extension at 72°C for 10 min followed. The second round was conducted in a reaction volume of 50  $\mu\text{L}$  and 5  $\mu\text{L}$  of the first-round product was used as the template. Reaction component concentrations were the same as in the first-round reaction with the following exceptions: the primers used were F3E (5' -CCTTC-CGCGCTTCGCTGCGT-3') and R4B (5' -CGTCTTCA-

AACCCCTACTG-3'), and the annealing temperature was 60°C. All reactions were performed in a GeneAmp PCR system 2400 (Applied Biosystems, CA). Amplified PCR product was purified by QIAquick gel extraction kit (QIAGEN). It was sequenced in both directions with a model Basestation automated sequencer (MJ Research Inc., MA) using an ABI Prism BigDye Terminator cycle sequencing kit (Applied Biosystems, CA) according to the manufacturer's instructions. Sequences were analysed with the BLAST® program (National Center for Biotechnology Information, Bethesda, MD).

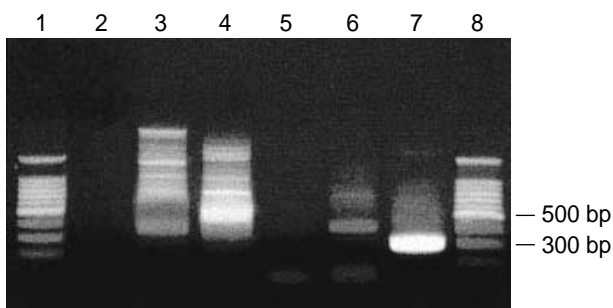


Fig. 2. Nested PCR product with template DNA extracted from the patient's fecal sample. Lanes 1 and 8, molecular size standard; Lanes 2-4: first-round PCR products; Lanes 5-7: second-round PCR products; Lanes 2 and 5, DNA of *Cryptosporidium parvum*; Lanes 3 and 6, *C. cayetanensis* negative human stool; Lanes 4 and 7, the patient's stool.

Round oocysts of about 8 to 9  $\mu\text{m}$  with wrinkle on the wall were found in modified acid fast stained stool specimen (Fig. 1A, B). Their stainability was variable from pale to red. Inside the oocyst wall, two sporocysts were observed by Normarski Differential Interference Contrast microscope (Olympus, Fig. 1C). The oocyst showed autofluorescence along the oocyst wall under UV illumination (Fig. 1D).

Multiple bands with various sizes were generated by using the F1E-R2B primer pair from DNA extracted from the fecal sample in the first-round PCR. About 294-bp fragment was amplified by F3E-R4B primer pair in the second-round PCR (Fig. 2). The amplicon was sequenced directly using primers F3E and R4B. The sequence (224-bp) obtained from the direct sequencing of the nested PCR product showed 100% homology with the sequence of 18S ribosomal RNA gene of *C. cayetanensis* registered in GenBank with accession numbers af111183 and u40261.

## DISCUSSION

There are several reports on intestinal cyclosporiasis among travelers returning from Indonesia (2, 4-7). In these reports, *Cyclospora* infections developed in immunocompetent individuals and provoked diarrheal illness, which persisted for several days with self-limitation. They also showed that *C. cayetanensis* was the main protozoal cause of the gastrointestinal illness and diarrhea in adult foreign residents during the wet season (November to May) in Indonesia (2). They insisted that unlike the *C. cayetanensis* infections among foreign residents of Jakarta, infections by this parasite in the indigenous population or in children are known to be rare.

In Korea, there has been no report of a case of domestic *Cyclospora* infection yet. Neither human outbreak nor environmental infection sources have been investigated. This is the first human case of *C. cayetanensis* reported in Korea as imported case from Indonesia. The patient was suspected to be infected by *C. cayetanensis* while she was traveling Indonesia based

on the facts that her diarrhea began while she was still in Indonesia, that she visited the country during the season, December, of high infection incidence, and the travel area was west Java known as a *Cyclospora* endemic zone. The source of infection and transmission route were not identified in this case, but contaminated water and vegetables have been suggested as an infection sources (8).

This case could have been neglected if the rest of the patient's stool had not been reexamined by the expert on parasite. While performing the epidemiological study on coccidian protozoa, we found the oocysts larger than those for *Cryptosporidium parvum* and showing variable stainability to modified acid fast staining. Because these two characteristics were enough to doubt *Cyclospora*, further study, i.e., wet mount under UV illumination and PCR, were carried out. Although we confirmed *Cyclospora* with PCR and direct sequencing in this study, the findings under modified acid fast staining would provide enough information for the routine diagnosis. The characteristics of the oocysts of *C. cayetanensis*, i.e., size (8 to 10  $\mu\text{m}$ ), variable stainability (red to pale), and autofluorescence under UV illumination, are the major differential points with those of *C. parvum* having smaller size (4 to 5  $\mu\text{m}$ ), consistent stainability (red), and no autofluorescence. For the uniform staining of *Cyclospora* oocysts, modified safranin technique was suggested by Visvesvara et al. (9).

This case shows that the clinical system for diagnosis of the cyclosporiasis is not adequate in hospitals in this country. As a matter of fact, there are very few places where special staining for *Cyclospora* or *Cryptosporidium* such as modified acid fast staining is established in the clinical diagnostic laboratory. In addition, physicians generally give a very little attention on *Cyclospora* as a cause of diarrheal illness yet. Probably these are the main reasons of no cyclosporiasis case has been reported in this country.

Recently the number of travelers to the tropics including Indonesia is increasing. Therefore it is highly recommended that the travelers should be informed of the endemic diseases, such as cyclosporiasis, in those areas, and health education for prevention is necessary before traveling. Also, physicians should be alert to the *Cyclospora* infection when they treat a patient complaining of diarrhea with a history of visiting to Indonesia, and an adequate diagnostic system for the *Cyclospora* infection should be implemented in clinical laboratories as soon as possible.

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