Gut pathogens, including parasites, are the leading cause of infections leading to enteric illness, and the predominant pathogenic organisms may vary temporally and spatially [1]. *Cyclospora cayetanensis* among these diverse gut pathogens has emerged as one of the most common apicomplexan protozoan causing chronic diarrhea illness worldwide [2]. *C. cayetanensis* initially known as coccidian-like body, large *Cryptosporidium* or small isospora-like protozoan. Like other diarrhea-causing pathogens, *C. cayetanensis* mainly causes acute watery diarrhea and abdominal pain, nausea and low grade of fever. Species of genus Cyclospora is known as host-specific. The human is known to only hosts of *C. cayetanensis* and become infected by ingesting food or water contaminated by oocysts. Moreover, this pathogen has been associated with cases of international traveler's diarrhea [3]. Epidemiologically, it is seasonal worldwide, even though there are regional changes. Its main diagnostic methods were oocyst detection with modified acid-fast staining by microscopy and molecular detection by PCR in fecal samples [4]. Moreover, also, this pathogen has been associated with cases of international traveler's diarrhea globally [5,6].

In recent years, this pathogen has been increasingly observed both in developed countries, such as the United States of America, United Kingdom and Canada [7-9], and in developing countries such as India, Nepal, Papua New Guinea, Vietnam, and Indonesia [7,10-14]. Especially, Nepal is one of the South-Asia countries with highly prevalent of intestinal pathogens and showed a higher prevalence of *C. cayetanensis* in drinking water, sewage water, green leafy vegetables, and school children [15-18]. In Korea, only one imported case with cyclosporiasis was reported from an individual infected after visiting Indonesia [19]. We report here the first case of a group infection with *C. cayetanensis* from a traveler visiting Nepal, and the second report of a traveler's diarrhea by *C. cayetanensis* imported in Korea.

**Abstract:** *Cyclospora cayetanensis* is an apicomplexan protozoan and is one of the most common pathogens causing chronic diarrhea worldwide. Eight stool samples with diarrheal symptom out of 18 Korean residents who traveled to Nepal were obtained, and examined for 25 enteropathogens including 16 bacterial species, 5 viral species, and 4 protozoans in stool samples as causative agents of water-borne and food-borne disease. Only *C. cayetanensis* was detected by nested PCR, and 3 PCR-positive samples were sequenced to confirm species identification. However, the oocysts of *C. cayetanensis* in fecal samples could not be detected by direct microscopy of the stained sample. As far as we know, this is the first report of a group infection with *C. cayetanensis* from a traveler visiting Nepal, and the second report of a traveler's diarrhea by *C. cayetanensis* imported in Korea.

**Key words:** Cyclospora cayetanensis, traveler's diarrhea, protozoa, PCR, Korea
After consultation of the first patient, additional stool samples were collected from her 7 colleagues with consent to epidemiological investigation about 5 days later. According to Guideline for Water-borne and Foodborne Diseases Control in KCDC, a total of 25 species pathogens, 16 bacterial species including Vibrio cholerae, Shigella spp, and pathogenic Escherichia coli, 5 viral species including Rotavirus and Norovirus, and 4 protozoans including Giardia lamblia and Cryptosporidium parvum/hominis, were targeted to examine [20]. The bacterial and viral pathogens were tested by the Public Health and Environment Research Institute (PHERI) in Gangwon-do, and protozoans were examined by the Division of Vectors & Parasitic Diseases (DVDP), KCDC. To detect water-borne pathogens from the stool samples, all stool samples were smeared on glass slides using the formalin-ether method and stained with modified acid-fast staining (MAFS) under microscopic observation for 3 times repeating the examination. Moreover, molecular detection method, nested PCR was performed for 4 protozoan parasite detection (Cryptosporidium parvum/hominis, Giardia lamblia, Entamoeba histolytica, C. cayetanensis). As a result, C. cayetanensis was identified in 3 out of 8 patients through nested PCR and sequencing analysis.

In Korea, the first case of cyclosporiasis causing traveler’s diarrhea was reported in 2003 in a patient who had traveled to Indonesia [19]. Similarly, in the present case, 8 of the 18 Korean travelers who visited Nepal together on April 1–9, 2013, had enteric symptoms, including diarrhea, upon their return to Korea, and a total of 8 stool samples from 8 patients were collected around April 20. Both Indonesia and Nepal are known as endemic regions of C. cayetanensis, so individuals may be suspected of being unknowingly exposed to C. cayetanensis while traveling. Common symptoms of C. cayetanensis infection are characterized by anorexia, watery diarrhea, fatigue, and low-grade fever; however, it can also be asymptomatic [4]. These and previous cases were similar in that they had diarrhea for 3 to 5 days after returning home as major clinical symptoms. They have all clinical symptoms like diarrhea (75%, 6/8), emesis (12.5%, 1/8), fever (12.5%, 1/8), and stomachache (25%, 2/8) (Table 1). However most of them did not visit a local hospital except 1 patient who visit a clinic at one time.

At the present investigation, any virus and bacterial microbes were not detected in all fecal samples through laboratory testing, whereas only the specific gene for C. cayetanensis was detected in 3 out of the 8 samples by nested PCR, showing a 294-bp fragment amplification of the 18S rRNA gene of the pathogen, F3E (‘5′-CCTCCCGCTCCTCCGTGCTC-3′) and R4B (‘5′-CGTCCTCAACCCCTCCTACTG-3′), and the annealing temperature was 60°C, following PCR procedure to the previous report [19] (Fig. 1). Using the sequencing data, phylogenetic trees were constructed using the CLUSTAL W multiple sequence alignment computer program (Histon, Cambridgeshire, UK) and the Molecular Evolutionary Genetics Analysis (MEGA) program; the robustness of groupings was assessed using 1,000 bootstrap replicates of the data. In comparative sequencing analysis of 3 PCR positive cases, all sequences showed 98% genetic identity with C. cayetanensis 18S rRNA and showed high similarity to sequences derived from 2 case individuals in Nepal (Accession No. U40261, AF111183) (Fig. 2) [21]. However, unfortunately, it could not be detected any oocysts of C. cayetanensis in all MAFS-stained samples by direct microscopy examination.

In the first outbreak of cyclosporiasis reported in Korea, the early stage stool samples isolated from patients showing diarrhoeal symptoms were collected in hospital returning to Korea; thus, oocysts of C. cayetanensis could be possible to more readily

### Table 1. Clinical symptoms of 8 Korean travelers upon their return from Nepal

| Sample No. | Sex   | Age (yr) | Symptoms exhibited | Diarrhea | Emesis | Fever | Stomachache | Self-medicating |
|------------|-------|----------|-------------------|----------|--------|-------|-------------|-----------------|
| 1          | Female| 52       | Y                 | Y        | -      | -     | -           | Y               |
| 2          | Male  | 57       | Y                 | Y        | -      | -     | -           | Y               |
| 3          | Male  | 61       | Y                 | Y        | -      | -     | -           | N               |
| 4          | Female| 50       | Y                 | -        | Y      | -     | Y           | N               |
| 5          | Male  | 46       | Y                 | Y        | -      | Y     | N           |                 |
| 6          | Female| 55       | Y                 | -        | -      | -     | -           | unknown         |
| 7          | Female| 56       | Y                 | Y        | -      | -     | Y           |                 |
| 8          | Female| 46       | Y                 | Y        | -      | -     | N           |                 |

*1 patient only visited a local clinic; Y, yes; N, no.
detectable [14]. In the present case, the period from occurring clinical symptoms to collecting time of stool samples took about 30 days. Oocysts can intermittently or continuously continue to be shed by non-symptomatic people, and symptoms can also persist in the absence of oocysts in faeces [22]. Thus the reason for the undetectable oocysts in the stool samples may be that they got over illness and clinical symptoms of most of the affected them had disappeared at the time of sample collection.

The prevalence of traveler’s diarrhea of unknown cause among individuals visiting Asia is estimated at 10-56% [1]. Especially, traveler’s diarrhea was the most common clinical symptoms during travel or on arrival and ongoing symptoms were showed by 25% of all travelers on return home [23]. Recently, tourism to Southeast Asian countries such as Nepal and Indonesia as well as Latin America has increased in Korea; thus, the incidence of traveler’s diarrhea caused by enteric pathogens including C. cayetanensis was possible to be increasing unofficially. Therefore, travelers experiencing continuous diarrhea after returning to home from South and Southeast Asia should consider the possibility that they could have been exposed to enteropathogenic parasites while traveling. In this case, C. cayetanensis infection is believed to be through local food intake. As a result of an epidemiological survey over the phone, it was found that the tour group visited the local church and consumed vegetables, water, and fruits. To prevent such infections, all travelers should maintain high standards of personal hygiene and have attention to eating some kinds of local foods while traveling. In addition, travelers with diarrhea of unknown cause after returning home should be appropriately examined at a healthcare facility and be continuously monitored.

The current case represents the second report of imported cyclosporiasis causing traveler’s diarrhea in Korea and the first report of a group infection with C. cayetanensis after visiting Nepal known as endemic country with cyclosporiasis.

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CONFLICT OF INTEREST

We have no conflict of interest related to this work.

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