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〈Short communication〉

Seasonal Dynamics of Pathogenic Microorganisms (*Cryptosporidium, Giardia* and Fecal Bacteria) in an Artificial Lake Ecosystem (Sangsa Lake, Korea)

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ABSTRACT: This study was performed for the purpose of monitoring monthly levels of two pathogenic microorganisms, *Cryptosporidiu*m and *Giardia*, from November 2005 to August 2007 in Sangsa Lake. Water temperatures, pH and DO fluctuated seasonally at the study site. Annual mean values of BOD, COD and SS were 0.8 mg L^{-1}, 2.3 mg L^{-1} and 1.9 mg L^{-1}, respectively. Although there was distinct seasonal variation in water chemistry and chlorophyll a concentration, the lake generally contains low concentrations of nutrients and chlorophyll a. The relative abundance of coliform bacteria was always greater than that of fecal coliform. The fecal coliform bacteria comprised 8.5～22.1% of total coliform bacteria. Seasonal analysis of *Cryptosporidiu*m and *Giardia* levels in the study site showed that in winter (November through February), *Cryptosporidium* oocysts and *Giardia* cysts were most abundant (1.1～1.8 × 10 cells L^{-1} and 3.8～5.1 × 10 cells L^{-1}, respectively), while in summer (July through September) the abundance was lowest (0.0～0.3 × 10 cells L^{-1} and 0.9～2.9 × 10 cells L^{-1}, respectively). Molecular identification revealed two subtypes of *Cyrptosporidium parvum* in Sangsa Lake.

Key words: *Crytosporidium*, *Giardia*, Pathogenic microorganisms, Sangsa lake

INTRODUCTION

Cryptosporidium and *Giardia* are major pathogenic protozoa that originate from fecal pollution in rivers or lakes (Fayer et al. 2000, Thompson et al. 2000, Caccio et al. 2005). These two parasitic protozoa cause cryptosporidiosis and giardiasis diseases, which can be lethal to people who are immunocompromised. It is well-known that *Cryptosporidium parvum* oocysts are more resistant than *Giardia lamblica* cysts to removal and inactivation by conventional water treatment such as coagulation, sedimentation, filtration, and chlorine disinfection. Accordingly, it is more difficult to remove them than other pathogens such as *Salmonella* and *Shigella* by conventional water treatment (Lee et al. 2000, Betancourt and Rose 2004).

Extensive studies have been performed in America, England, and Japan after reported outbreaks of these protozoa-caused diseases in the 1980s (Craun et al. 1998, Clancy et al. 1999). In the U.S.A., the Safe Drinking Water Act Amendments of 1966 require the U.S. Environmental Protection Agency (EPA) to evaluate the risk to public health posed by drinking water contaminants, including waterborne parasites such as *Cryptosporidium*. However, infectious diseases caused by *Cryptosporidium* and *Giardia* have rarely been reported in Korea (Lee et al. 2000) and little information on protozoa in water resources is available. The objective of this study was to evaluate the seasonal occurrence of pathogenic microorganisms in an artificial lake system, focusing on the dynamics of *Cryptosporidium* and *Giardia* populations. A suite of environmental and bacteriological parameters was also evaluated in order to elucidate possible factors affecting the dynamics of pathogenic microorganisms in the system.

MATERIALS AND METHODS

Site Description and Sample Collection

Sangsa Lake is located in the south-western part of South Korea (～ 34° 56' 54" N and 127° 24' 59" E) and has a total catchment area of 134.6 km^2, a surface area of 7.8 km^2, and a water capacity of 25×10^7 m^3 (Fig. 1). Lake water is primarily used for agricultural purposes such as irrigation and as drinking water. Water samples were monthly collected from a site at 34° 56' 5.16" N and 127° 25' 2.09" E on Sangsa Lake (at a 0.5 m depth) from November 2005 to August 2007 and kept in the shade at ambient temperatures for up to 1 hr until they were transported to the laboratory for analysis.

Water Quality Analysis and Bacterial Enumeration

All analyses of basic limnological variables (e.g., water tempera-

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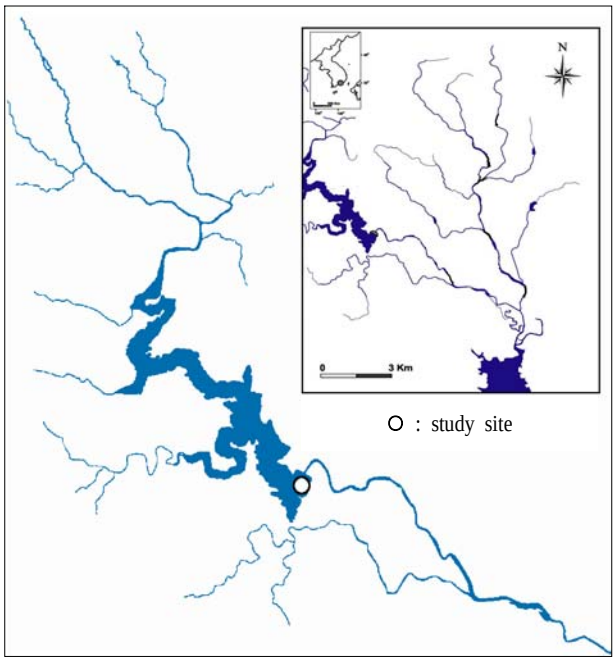


Fig. 1. Map showing the study site (○ : sampling site). Water samples were collected from a site at 34° 56' 5.16" N and 127° 25' 2.09" E on Sangsa Lake from a depth of 0.5 m.

ture, pH, DO, BOD, COD, SS, T-N, T-P, NO_3-N, NH_3-N, PO_4-P, chlorophyll a) followed standard methods (Wetzel and Likens 1991). Bacteriological examinations were also performed for both coliform and fecal coliform bacteria from September 2006 to August 2007. The number of bacteria was enumerated by the Most Probable Number (MPN) method (Alexander 1982).

Analytical Procedures for Detection of *Cryptosporidium* and *Giardia*

We measured the recovery rate using negative and positive controls. For the positive control, water samples collected from Sangsa Lake each month for the 22 months of the study were inoculated with the standard inoculation solution, Easyseed™ ("Easyseed 100", BTF, *Crytospridium* 100 ± 2.4, *Giardia* 99 ± 2.0) purchased from BTF Precise Microbiology (Sydney, Australia), using the USEPA 1623 method (USEPA 1999). *Cryptosporidium* and *Giardia* strains were isolated from water samples of at least 10 L with anti-*Cryptosporidium* and anti-*Giardia* magnetic beads (Dynabeads Combokit, Danal 730.02) using the immunomagnetic separation method. The purified *Cryptosporidium* oocysts or *Giardia* cysts were then immunoblotted using FITC-labeled anti-*Cryptosporidium* or anti-*Giardia* monoclonal antibodies (Direct labeling kit, Merdian Co.), and applied to a DAPI (4',6-diamidino-2-phenylindole) solution [1/5,000 diluted solution of a mixture of 50 mL 150 mM PBS plus 10 μL of DAPI (2 mg mL^{-1})] for staining. Identification and enumeration were performed on a Zeiss™ Axioskop fluorescent microscope (Carl Zeiss, Inc., USA). 18S rRNA-based molecular detection of *Cryptosporidium* and *Giardia* was performed using two sets of PCR primers: 18sif-18sir (5'-AGT GAC AAG AAA TAA CAA TAC AGG; 5'-CCT GCT TTA AGC ACT CTA ATT TTC for *Cryptosporidium*) and GDHeF(or GDHiF)-GDHiR (5'-TCA ACG TYA AYC GYG GYT TCC GT or 5'-CAG TAC AAC TCY GCT CTC GG; 5'-GTT RTC CTT GCA CAT CTC C for *Giardia*) (Miller et al. 2005).

RESULTS

Water Quality and Bacteria Abundance

Water temperatures ranged from 5～23℃ from November 2005 to August 2007, exhibiting extensive seasonal variation. pH and DO also fluctuated seasonally, ranging from 7.2 to 8.2 and from 6.7 to 14.9, respectively. Annual mean values of BOD, COD and SS were 0.8 mg L^{-1}, 2.3 mg L^{-1} and 1.9 mg L^{-1}, respectively. There was distinct seasonal variation in water chemistry, although the lake generally contains low concentrations of nutrients (Table 1). One of the most dramatic seasonal changes was in chlorophyll a concentration. Chlorophyll a concentration was high in early July 2006. The average chlorophyll a concentration was 3.37 μg L^{-1} (range =1.2～14 μg L^{-1}).

The abundance of coliform bacteria was always greater than that

Table 1. Means, standard deviations, and ranges of limnological parameters in the study site in November 2005～August 2007 (n = 21)

Parameters	Unit	Study site
Water temperature	℃	13.2± 6.1 (5.0～23.0)
pH		7.6± 0.2 (7.2～ 8.2)
Dissolved oxygen	mg L^{-1}	11.1± 2.4 (6.7～14.9)
BOD	mg L^{-1}	0.8± 0.2 (0.6～ 1.5)
COD	mg L^{-1}	2.3± 0.2 (1.7～ 2.6)
SS	mg L^{-1}	1.9± 0.9 (0.7～ 4.3)
Chl. a	μg L^{-1}	3.3± 2.6 (1.0～14.0)
TN	mg L^{-1}	1.0± 0.1 (0.8～ 1.4)
TP	μg L^{-1}	12.0± 5.0 (4.0～29.0)
NH_4^+-N	μg L^{-1}	18.0±16.0 (1.0～56.0)
NO_3^--N	mg L^{-1}	0.6± 0.1 (0.5～ 0.8)
PO_4^{3-}-P	μg L^{-1}	2.5± 1.2 (1.0～ 5.0)

of fecal coliform. The fecal coliform bacteria comprised 8.5～22.1% of total coliform bacteria (Fig. 2). Bacteriological examination detected coliform bacteria at a mean frequency of 159 MPN 100 mL^{-1} (range = 79～350 MPN 100 mL^{-1}) and fecal coliform bacteria (which are the indicator for fecal pollution) at a mean frequency of 16.6 MPN 100 mL^{-1} (range = 7.8～33 MPN 100 m L^{-1}).

Seasonal Dynamics of *Crytosporidium* Oocysts and *Giardia* Cysts

Crytosporidium oocysts and *Giardia* cysts were found at a frequency of 0～4.7 × 10 cells L^{-1} and 0～1.8 × 10 cells L^{-1}, respectively (Fig. 3). The highest levels of *Crytosporidium* and *Giardia* were 1.8 × 10 cells L^{-1} and 5.1 × 10 cells L^{-1}, respectively, in February 2006. In spring and summer, the levels tended to decrease. In summer, the level of *Giardia* was below 1 × 10 cells L^{-1}, suggesting little pollution with *Giardia*. Beginning in autumn, the abundance of *Crytosporidium* and *Giardia* tended to increase.

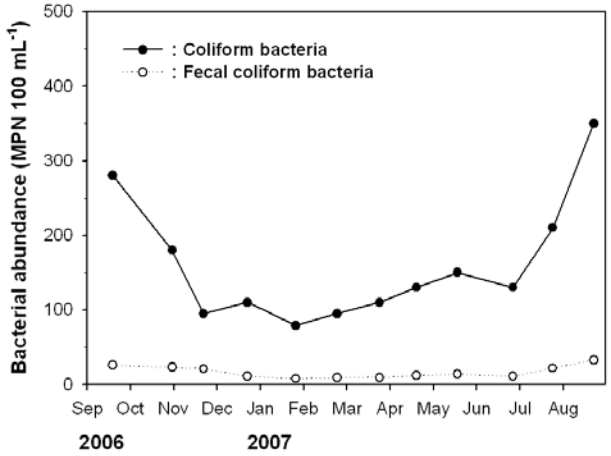


Fig. 2. Seasonal changes in coliform and fecal coliform bacterial abundance (MPN 100 mL^{-1}).

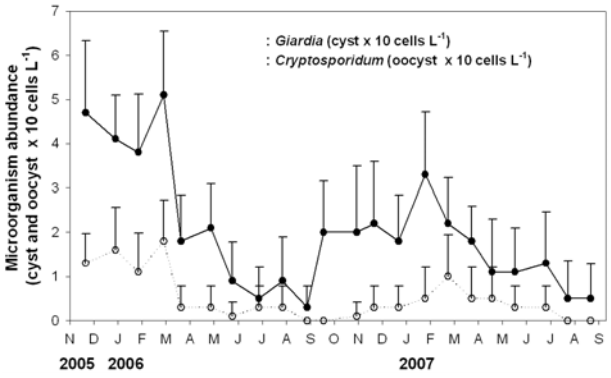
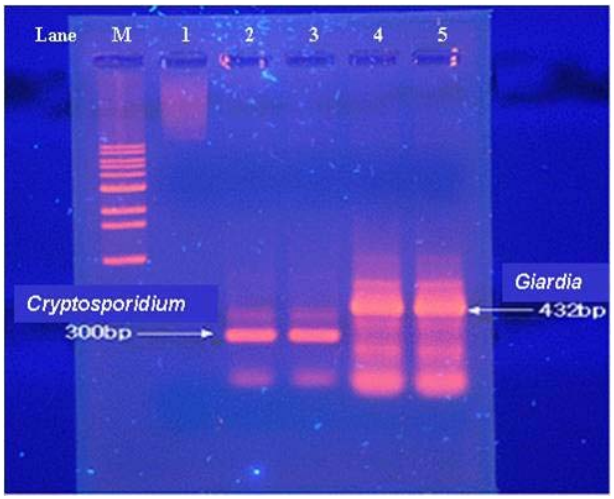


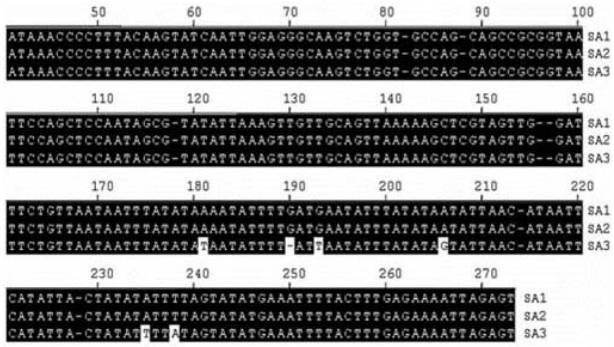
Fig. 3. Seasonal changes in *Cryptosporidium* (oocsyt × 10 cells L^{-1}) and *Giardia* (cyst × 10 cells L^{-1}) abundance (mean±s.d., n = 10).

Detection of *Cyrptosporidium* Based on 18S rRNA

Water samples containing *Cyrptosporidium* oocysts and *Giardia* cysts were transferred into separate PCR tubes following microscopic isolation, and semi-nested PCR was performed. All samples showed specific amplification products of approximately 300 bp for *Cyrptosporidium* and 430 bp for *Giardia* on an agarose gel (Fig. 4a). A 273 bp sequence of the amplified *Cyrptosporidium* 18S rDNA was 98% similar to the corresponding sequence in the *C. parvum* 18S rRNA gene (accession no. EF175936) (Fig. 4b). However, the selected subclones which were assumed to be *Giardia* genomic DNA exhibited no similarity with that found in the NCBI nucleotide database.



(a)



(b)

Fig. 4. (a) Gel showing PCR amplification of 18S rRNA of *Cryptosporidium* and *Giardia*, (b) sequence alignment of 18S rRNAs of *Cryptosporidium* strains. In panel (a), M, size marker, λ-*Hin*d III; lane 1, water sample DNA; lane 2～3, 18S rRNA PCR product for *Cryptosporidium*; lane 4～5, 18S rRNA PCR product for *Giardia*. 50 ng of water sample DNA was used for the amplification of 18S rRNAs from *Cryptosporidium* and *Giardia*. In panel (b), the sequence of clone SA1 18S rRNA was the same as that of clone SA2.

DISCUSSION

This study examined the seasonal dynamics of *Crytosporidium*, *Giardia*, and coliform bacteria in an artificial lake (Sangsa Lake, Korea) from November 2005 to August 2007, and performed quality control and assurance to establish the detection efficiency for protozoans and to establish appropriate analytical techniques. The control quality test using the EPA 1623 method showed that recovery rate was highly variable, ranging from 34～70% for *Crytosporidium* and 38～78% for *Giardia*.

The MPN-based bacteriological examination placed the water quality of Sangsa Lake between Grade Ia (80 MPN 100 mL^{-1}) and Grade Ib (1000 MPN 100 mL^{-1}) according to the guidelines of the Korean Ministry of Environment. Based on fecal coliform bacteria, the water in Sangsa Lake was Grade Ia (10 MPN 100 mL^{-1}) in the winter, while in summer the water quality was between Grade Ia (10 MPN 100 mL^{-1}) and Grade Ib (100 MPN 100 mL^{-1}). These observations suggest there may be a negative relationship between occurrence of bacteria and parasitic protozoa related to seasonal factors, even though the occurrence frequencies for both were below the levels specified by the Korean Ministry of Environment.

In this study, *Crytosporidium* and *Giardia* exhibited similar seasonal variation in their occurrence. Both protozoa were detected at the highest abundance in winter, while in summer the abundance was lowest, which is consistent with the results of previous studies (Jakubowski et al. 1996, Atherholt et al. 1998, Di Giovanni et al. 1999). A previous study reported that *Giardia* cysts could survive for 56～84 days in lakes in winter (DeRegnier et al. 1989). Sykora et al. (1991) also reported that survival of *Giardia* was negatively correlated with water temperature, while Craun et al. (2006) suggested that *Crytosporidium* and *Giardia* may be more abundant in winter due to a combination of higher survivorship in low temperatures and a decrease in the precipitation rate. The pronounced increase in rainfall in summer might also reduce the protozoan concentration. Our results also suggested that low temperatures in the winter might increase the abundance of *Crytosporidium* and *Giardia*. In contrast, coliform and fecal coliform bacteria were found to increase in abundance in summer when water temperature increases.

This study showed that two major pathogenic protozoa occur in Sangsa Lake, as in other lakes or rivers. Our data are the first to valuate the quality of water resources in Sangsa Lake based on pathogenic protozoa. However, the short-term nature of our investigation makes it difficult to determine the overall water quality of Sangsa Lake based on our data. Therefore, a longer-term examination will be necessary to determine the overall effect of the pathogenic protozoa, *Cryptosporidium* and *Giardia*, on water quality. The EPA 1623 method used in this study had an enhanced detection rate and decreased the rate of false positive results. However, the IMS (immunomagnetic separation method) is limited in its ability to determine viability and infectiousness of the two protozoa.

A molecular study confirmed that *Crytosporidium* and *Giardia* occurred in Sangsa Lake (Fig. 4). Sequence analyses of 18S rRNAs from both protozoa placed the *Cryptosporidium* strains from Sangsa Lake in the species, *Cyrptosporidium parvum*, while *Giardia* clones were not obtained in spite of the presence of PCR product. Sequence analysis of 18S rRNA sequences from twenty five clones of *Cyrptosporidium* found in the water samples placed twenty clones in *C. parvum* type SA1 (the 18S rRNA sequence for type SA2 is identical to that of SA1) and five clones in type SA3 (Fig. 4b). Therefore, only two subtypes of *C. parvum* were identified in this study. This limited variation might be due to the very low occurrence of *Cryptosporidium* in Sangsa Lake or to the use of a small number of water samples. Further intensive studies of Korean types of *Cryptosporidium* and *Giardia* are clearly warranted.

ACKNOWLEDGEMENTS

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