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▣ BRIEF COMMUNICATION

High Prevalence of *Enterobius vermicularis* Infection among Schoolchildren in Three Townships around Yangon, Myanmar

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Abstract: In order to determine the status of *Enterobius vermicularis* infection among schoolchildren in suburban areas of Myanmar, 761 primary schoolchildren in 3 different townships around Yangon City were subjected to a survey using cello-tape anal swabs. The subjected schoolchildren were 383 boys and 378 girls who were 5-7 years of age. Only 1 anal swab was obtained from each child. The overall egg positive rate of *E. vermicularis* was 47.2% (359 positives), and sex difference was not remarkable (48.6% in boys and 45.8% in girls). However, the positive rate was the highest in South Dagon (54.6%) followed by Hlaing Thayar (43.8%) and North Dagon (34.8%). This difference was highly correlated with the living standards of the people in each township. Nucleotide sequence of the 5S rDNA from the eggs on the cello-tape (2 children) revealed 99.7% identity with that of *E. vermicularis* reported in GenBank. The results indicated that *E. vermicularis* infection is highly prevalent among primary schoolchildren around Yangon, Myanmar.

Key words: *Enterobius vermicularis*, enterobiasis, high prevalence, anal swab, schoolchildren, Myanmar

Intestinal helminthiases, in particular, soil-transmitted and contact-borne helminth infections are the most common parasitic infections in tropical and subtropical countries [1]. No less than a billion people are infected with at least one species of intestinal helminth [1]. The pinworm, *Enterobius vermicularis*, is the most common helminth of humans and has a worldwide distribution including even developed countries such as Western Europe and United States. The estimated global population infected by the pinworm is about 4-28% [1]. The common mode of pinworm transmission is ingestion of eggs either directly or indirectly through hands, bedding, clothing, and toilet seats [2,3]. Enterobiasis is commonly asymptomatic; however, children with high parasitic burdens have impairments in physical, intellectual, and cognitive development [4].

Until present, there have been few documented reports on the prevalence of *E. vermicularis* among people in Myanmar. A Thai research group reported that the egg positive rate of *E. vermicularis* among 372 immigrant children from Myanmar who lived in Samut Sakhon Province, Thailand was 25.2% [5]. No other reports are available. Thus, in the present study, we performed a small survey on *E. vermicularis* infection targeting primary schoolchildren in 3 different townships located around Yangon City, Myanmar in 2014.

The survey target included 7 primary schools (Fig. 1) in 3 suburban areas (South Dagon, Hlaing Thayar, and North Dagon) of Yangon. The subjects were 761 primary schoolchildren (383 boys and 378 girls) who were 5-7 years of age. The cellophane tape (cello-tape) anal swab method was used to detect the eggs. The sticky side of the transparent cello-tape was stuck to each child's perianal area and removed. Collected samples were transported to the National Health Laboratory, Yangon, Myanmar and examined under light microscopy by at least 2 medical specialists. The prevalence of *E. vermicularis* eggs was compared between sex and township, and the results were statistically evaluated by the chi-square test.

For identification of the pinworm species, PCR and nucleo-

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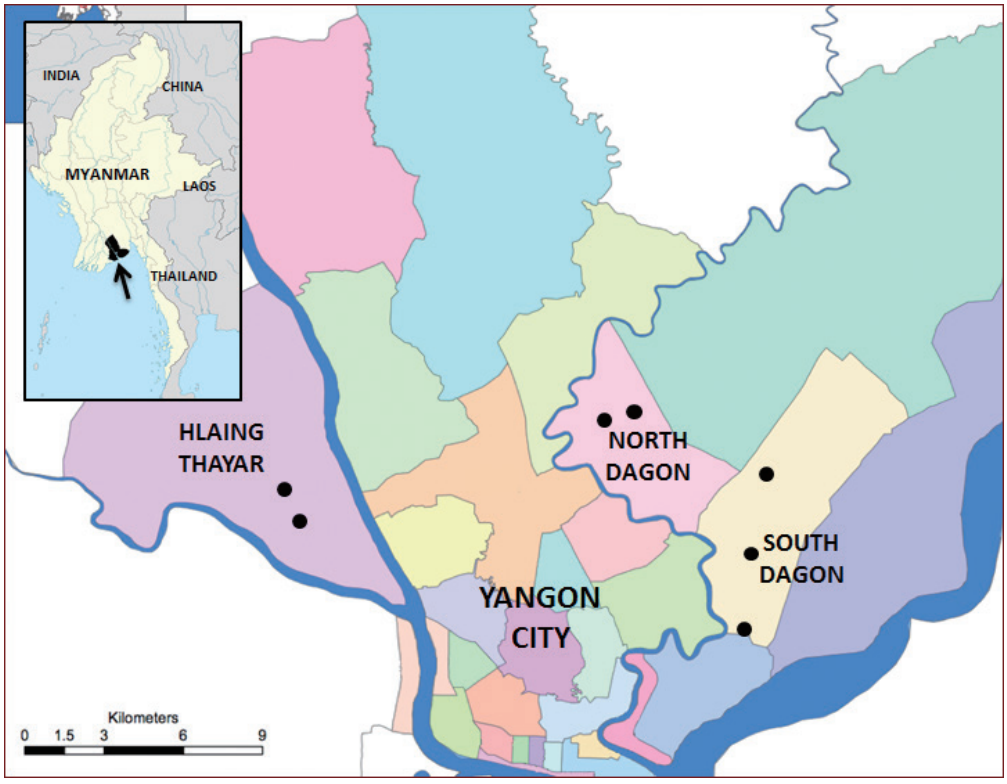

Fig. 1. Map showing the surveyed areas around Yangon City, Myanmar. The schoolchildren of 7 primary schools (black dots) in 3 communes (South Dagon, Hlaing Thayar, and North Dagon) were subjected in this study.

Table 1. The egg positive rate of *E. vermicularis* among primary schoolchildren in Yangon, Myanmar by cello-tape anal swab examination in 2014

Area	Male			Female			Total		
	No. of exam.	No. of positive	(%)	No. of exam.	No. of positive	(%)	No. of exam.	No. of positive	(%)
South Dagon	199	113	56.8	193	101	52.3	392	214	54.6[a]
Hlaing Thayar	92	41	44.6	93	40	43	185	81	43.8[a]
North Dagon	92	32	34.8	92	32	34.8	184	64	34.8[a]
Total	383	186	48.6	378	173	45.8	761	359	47.2

[a]The egg positive rate was significantly higher ($P<0.05$) in South Dagon than in Hlaing Thayar and North Dagon.

tide sequencing were performed on the 5S rDNA region according to the procedures reported previously [6]. Total genomic DNA was extracted from the eggs on the cello-tape by using a DNeasy Blood and Tissue Kit (QIAGEN, Hilden, Germany) with minor modifications. Briefly, the eggs were mechanically detached from the cello-tape using forceps under a stereomicroscope, and the isolated eggs were vortexed with glass beads for lysis. The next step followed the manufacturer's instructions. The PCR product was amplified by using the Cosmo Labopass X2 PCR Premix kit (Cosmo Genetech, Seoul, Korea) with primers of 5S rDNA (forward primer 5'-CACTTGCTATACCAACAACAC-3'; reverse primer 5'-GCGCTACTAAACCATAGAG-3'), and automated DNA sequencing was performed by Solgent Co. (Daejeon, Korea). Nucleotide sequences obtained were aligned by using the Geneious Program, version 7.1.7 (Geneious Co., Wellington, New Zealand) [6].

The *P*-values of <0.05 were considered statistically significant. This study was approved by the National Health Laboratory, Yangon, Myanmar and the Korea Association of Health Promotion, Korea under the agreement of Korea-Myanmar International Project on Intestinal Parasite Control in Primary Schoolchildren around Yangon, Myanmar (2013-2015).

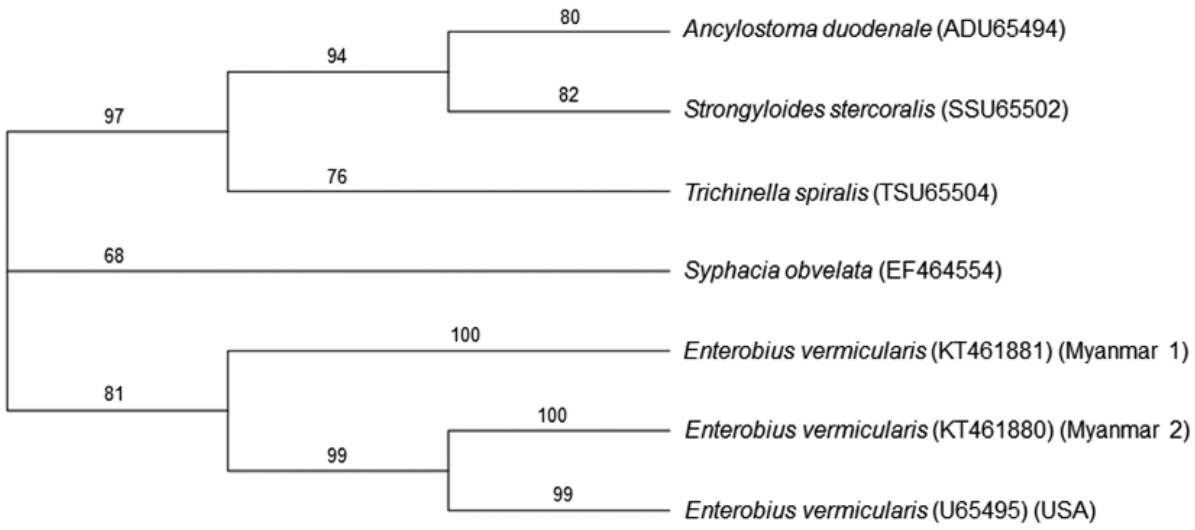

Fig. 2. A phylogenetic tree based on 5S rDNA gene sequences exploring the relationships of our specimens (Myanmar 1 & 2) with ever-reported sequences in GenBank. Numbers above the branches are bootstrap values. The phylogenetic tree reveals that the 5S rDNA gene sequence from the eggs on cello-tape anal swabs of 2 schoolchildren from Myanmar is compatible with that of *E. vermicularis* (USA).

Out of the total 761 primary schoolchildren examined, 359 (47.2%) were positive for the eggs of *E. vermicularis* (Table 1). The positive rate was not significantly ($P>0.05$) different between boys (186/383, 48.6%) and girls (173/378, 45.8%). As the age of the examined schoolchildren were the same among most subjects, 5-7 years old, no age-specific different in the prevalence could be noted. However, the prevalence was significantly higher ($P<0.05$) in South Dagon (54.6%) than in Hlaing Thayar (43.8%) and North Dagon (34.8%) townships. The prevalence was the highest in boys from South Dagon (56.8%) and the lowest in boys and girls from North Dagon. Sequencing of the 5S rDNA gene of the eggs on the anal swabs showed 99.7% identity (identical in 341 among 342 sites) with *E. vermicularis* reported from USA (GenBank no. U65495). The neighbor-joining tree revealed that our 2 specimens (Myanmar 1 & 2, GenBank no. KT461881 and KT461880, respectively), were phylogenetically compatible to *E. vermicularis* (Fig. 2).

The parasite infection rate in specific population such as children and rural residents is particularly attributed to environmental and personal hygiene and sanitation [7,8]. For this reason, the primary schoolchildren living in South Dagon seem to live in a poorer socioeconomic status and worse environment than those of the other 2 areas with a higher risk for *E. vermicularis* infection. It is locally well known that the general household income and living standards of people in South Dagon is lower than those in Hlaing Thayar and North Dagon townships. Further studies regarding the relationships between the pinworm prevalence and the living standard of people around the surveyed areas in Myanmar should be studied in the near future. By this study, however, it can be concluded that all 3 surveyed areas around Yangon, Myanmar are highly endemic areas of enterobiasis among primary schoolchildren.

There is little information on the general status of intestinal helminth infections in Myanmar, and most surveys were small scale or local hospital-based [9]. Especially, no studies have been performed on the prevalence of *E. vermicularis* infection in Myanmar. In the present study, the egg positive rate (47.2%) of *E. vermicularis* was much higher than that reported in other countries; 6.0% [2], 10.5% [8], or 18.5% [3] in South Korea, 0.5% in Taiwan [10], and 12.1% in Turkey [11]. However, in a recent report from China, the egg positive rate of children in Guangdong area was as high as 54.9% [4], slightly higher than that observed in Myanmar in the present study. Interestingly, the egg positive rate of immigrant children from Myanmar who lives in Thailand was 25.2%, a much lower figure than that observed in the present study [5]. It can be speculated that the immigrant Burmese children residing in Thailand have a better socio-economic status and better environment than in their home villages in Myanmar.

There are 2 main points for effective management and control of *E. vermicularis* infection. First, medications should be in mass-treatment style and should be repeated in 2-3 weeks

[12,13]. The pinworms are easily reinfected, and medication does not kill all pinworm larvae or juvenile worms in the intestine [12]. Therefore, repeated dose is an important strategy to treat larval/juvenile infections which remained in the host intestine after 1 time medication. Second, Gungoren et al. [14] reported the effects of hygiene promotion on the risk of reinfection. According to their results [14], the hygiene promotion group had a much lower risk of reinfection than the treatment only group (no hygiene promotion) or control group (no hygiene promotion, no medication).

The reason for a molecular analysis on the eggs in our study was to rule out a possibility for the present pinworms to be a species different from *E. vermicularis*, for example, *Enterobius gregorii* Hugot, 1983 [15] and *Syphacia obvelata* (Rudolphi, 1802) Seurat, 1916 [16]. *E. gregorii* is a human-infecting pinworm species reported to be unique from *E. vermicularis* in the morphology of the spicule in male worms [15]. However, *E. gregorii* is considered a synonym of *E. vermicularis* by several other workers [17,18]. *S. obvelata* is a pinworm species infecting rodents but can also infect humans [16]. Our specimens showed a 99.7% homology in the 5S rDNA gene sequence with that of *E. vermicularis* in GenBank but distinct from the sequence of *S. obvelata*. The 5S rDNA gene sequence of *E. gregorii* is unavailable in GenBank.

Taken together, the results of the present study suggest strongly that there is an urgent need to control *E. vermicularis* infection among schoolchildren in the surveyed areas of suburban Yangon, Myanmar.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest related to this study.

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