

Case Report

Infectious Diseases,
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Ancient Soil-Transmitted Parasite Eggs Detected from the Sixth Century Three Kingdom Period Silla Tomb

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ABSTRACT

The parasitic infection patterns of the Joseon period have begun to be revealed in a series of paleoparasitological studies. However, parasitism prevailing during or before the Three Kingdom period is still relatively unexplored. In the present study, we therefore conducted parasitological examinations of soil and organic-material sediments precipitated upon human hipbone and sacrum discovered inside an ancient Mokgwakmyo tomb dating to the Silla Dynasty (57 BCE–660 CE). Within the samples, we discovered ancient *Ascaris lumbricoides* (eggs per gram [EPG], 46.6–48.3) and *Trichuris trichiura* (EPG, 32.8–62.1) eggs, the species commonly detected among Korean populations until just prior to the 1970s. These findings show that soil-transmitted parasitic infection among the Silla nobility might not have been uncommon. This is the first-ever report on the presence of ancient parasite eggs in the samples obtained from a Three Kingdom period tomb; and it also presents the earliest positive results for any of the ancient South Korean tombs paleoparasitologically examined to date.

Keywords: Silla Kingdom; Paleoparasitology; *Ascaris lumbricoides*; *Trichuris trichiura*

INTRODUCTION

Paleoparasitology is the parasitological study of ancient samples recovered at archaeological sites. For the past several decades, a significant body of paleoparasitological research has elucidated the patterns of parasitic infection in ancient societies worldwide, which information could not easily have been obtained by any other means.¹⁻³ Briefly, ancient *Ascaris* eggs discovered at an archaeological site in Grand Grotte, France could be traced back to 30,000 years BP.⁴ Also, several-thousand-year-old *Trichuris* eggs have been found in the samples obtained from Kruger Cave in South Africa.⁵ Indeed, the findings of ancient intestinal parasite eggs have been reported at a great variety of archaeological sites all over the world.⁶

In Korea too, the reports on ancient samples from various archaeological sites have been forthcoming. For example, parasitologists have confirmed that pre-20th century Korean populations were seriously infected by different kinds of parasites.⁷⁻¹¹ Most of these

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Disclosure

The authors have no potential conflicts of interest to disclose.

Author Contributions

Conceptualization: Shin DH. Data curation: Seo M, Chai JY. Investigation: Oh CS, Hong JH, Ju JO.

investigations have been made on the fecal samples from Joseon period (1392–1910 CE) mummies³ or soil-sediment samples of the geological strata of various archaeological sites.¹²

As for ancient tombs, however, especially Silla cases dating to or before the Three Kingdoms in Korean history, there have been very few parasitological reports. Considering Korea's long and intricately documented history, this data gap in the paleoparasitological records is disappointing. Therefore, the current report might particularly be meaningful to the researchers because it is the first-ever parasitological confirmation of the presence of ancient parasite eggs in a tomb dating to the Silla Dynasty prior to the 676 CE unification of the Korean peninsula.

CASE DESCRIPTION

In 2013, the Silla Cultural Heritage Research Institute investigated an archaeological excavation site at Gyo-dong, Gyeongju, Korea. The investigation had been officially permitted by the Cultural Heritage Administration of Korea (approval number: 2013-0285). In the course of the excavation, a Mokgwakmyo tomb (wooden chamber tomb) was discovered, within which a wooden coffin contained a number of human skeletons (Fig. 1A). The archaeological and anthropological progress on this case is summarized in our previous report.¹³

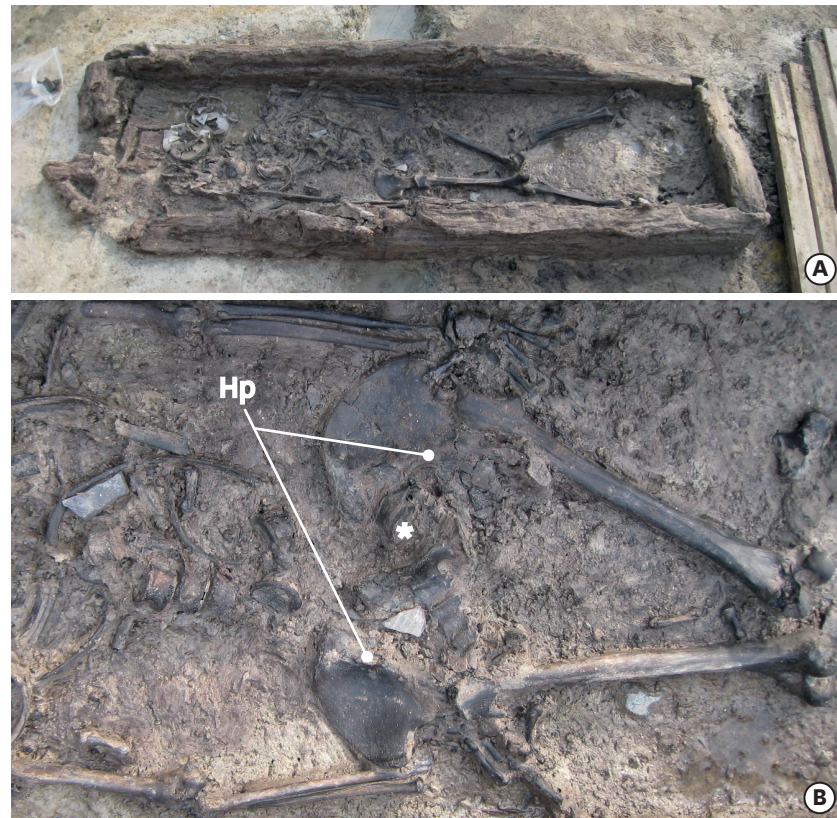


Fig. 1. Archaeological site at Gyo-dong, Gyeongju, Gyeongsangbuk-do province of Korea. (A) Sixth century Silla Mokgwakmyo tomb could be identified in the site. Human bones and multiple cultural remains could be also found within the coffin. (B) The soil-organic material precipitates were collected from the Hp or sacrum (indicated by asterisk). Hp = hip bones.

Analysis of cultural relics found within the coffin dated the tomb's construction to about the sixth century, thus locating it to the Three Kingdom era (57 BCE–668 CE) in Korean history. Soil and organic-material precipitates on the hipbones and sacrum were collected for a detailed parasitological examination (Fig. 1B).

Each soil and organic-material specimen (1–4 grams) was rehydrated in 0.5% trisodium phosphate solution with continuous shaking,¹⁴⁻¹⁷ filtered through multiple-layered gauze, and precipitated for one day. The precipitate thus obtained was re-dissolved in 0.5% trisodium phosphate solution (final volume, 20 mL). Solution slides of all of the specimens subsequently were examined under light microscopy (BH-2; Olympus, Tokyo, Japan).

In the samples (total, 200 μ L; 20 μ L each time; 10 times), we identified ancient *Ascaris lumbricoides* and *Trichuris trichiura* eggs (Fig. 2). The measured dimensions (mean \pm standard deviation) of the *Ascaris* and *Trichuris* eggs were 58.5 ± 2.8 (55.0–65.0) \times 47.6 ± 2.9 (45.0–52.5) μ m and 50.1 ± 0.7 (49.0–51.2) \times 24.1 ± 0.9 (23.0–26.0) μ m, respectively. The numbers of eggs per gram (EPG) ranged between 46.6 and 48.3 (*Ascaris*) and 32.8 and 62.1 (*Trichuris*) (Table 1).

DISCUSSION

In previous parasitological studies on archaeological specimens, we were able to partially reconstruct the parasite-infection prevalence patterns among pre-20th century Korean populations. For instance, the examinations of fecal samples obtained from Joseon mummies yielded ancient *Ascaris*, *Trichuris*, *Clonorchis*, *Paragonimus*, and *Metagonimus* spp. eggs.³ According to our forthcoming series of reports, the overall *Ascaris*- and *Trichuris*-positivity rates during the Joseon Dynasty were 58.3 and 83.3%, respectively.¹⁸

Data obtained from Joseon mummy fecal samples, particularly in comparison with 20th century national survey data on parasitic infection in Korea, can significantly further our understanding of parasitism in pre-20th century Joseon society. Interestingly, historical rates of soil-transmitted parasitic infection in Korea did not markedly differ until the late 20th century (54.9% for *Ascaris*, 65.4% for *Trichuris*; national survey of 1971).^{3,19}

As for the parasite-infection patterns prevailing prior to the Joseon period, however, the data remain scanty. In our previous paleoparasitological studies on samples obtained from archaeological sites representative of the Three Kingdom period or earlier, our positive findings on ancient parasite eggs were mostly confined to the soil samples of geological strata at ancient moats, reservoirs, or shell-middens.³ Such findings, though certainly suggestive of excreta origins, do not constitute any direct evidence of parasitic infection among Silla people. In this regard, the current examinations of soil and organic-material precipitate samples from the sixth century Silla Mokgwakmyo tomb can be considered integral to the researchers' efforts to reconstruct a complete, Korean-history-wide parasite-infection pattern.

Our discovery of *Ascaris* and *Trichuris* spp. eggs is the earliest among the ancient tombs for which we have found positive paleoparasitological results thus far. Since the individual found within the coffin was, judging from the status of the accompanying cultural remains, a member of the lower Silla nobility,¹³ this report also can be the first direct evidence of soil-transmitted parasitic infection among Three Kingdom period nobility. Considering the

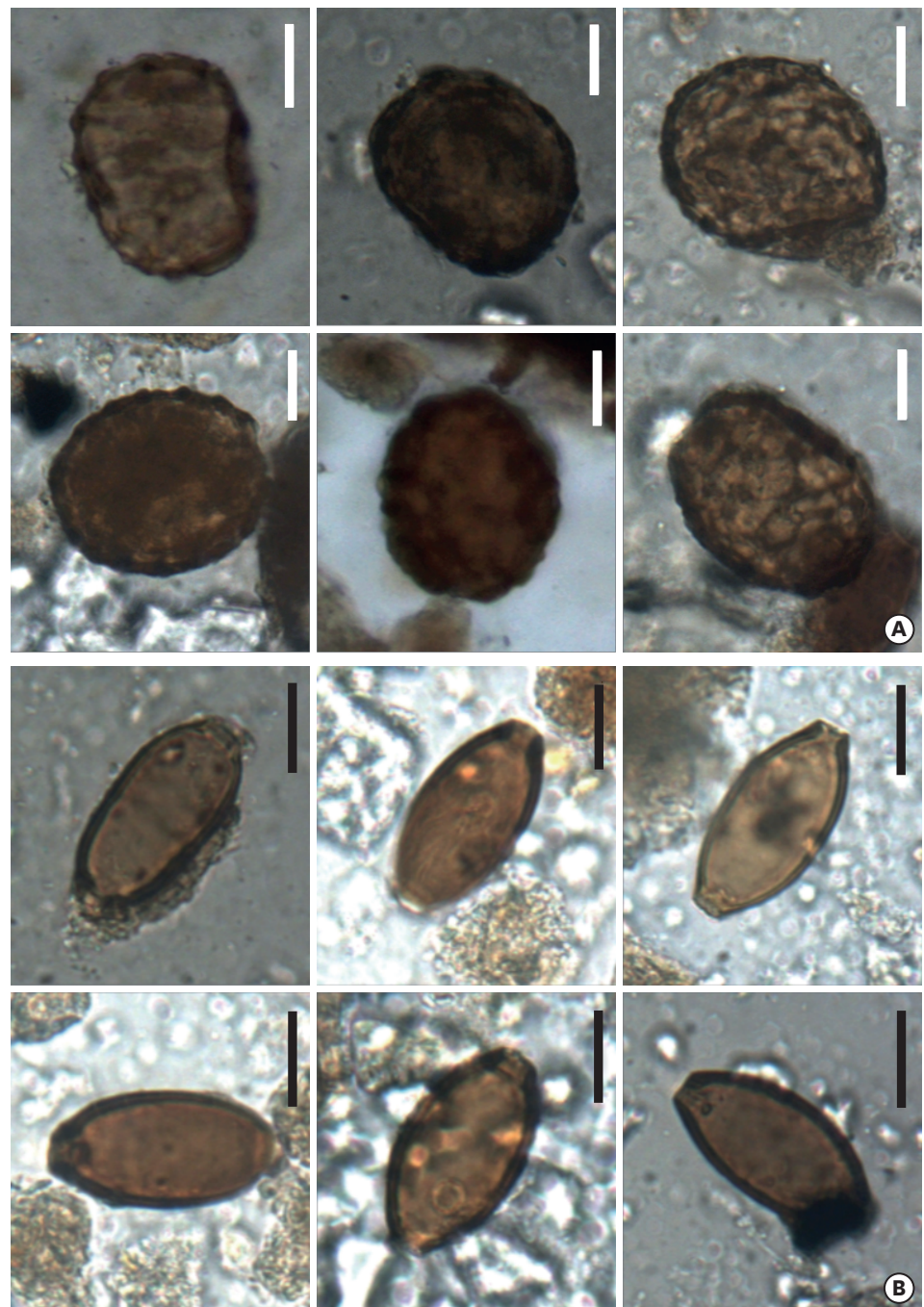


Fig. 2. Ancient parasite eggs observed in the sixth century Silla Mokgwakmyo tomb samples. **(A)** *Ascaris lumbricoides* and **(B)** *Trichuris trichiura* eggs were found. Scale bars = 20 μ m.

Table 1. Results of paleoparasitological examinations in this study

Archaeological information	Sampling location at the site	Parasite eggs identified	Range of dimensions, μ m	EPG
6th century Silla Mokgwakmyo tomb at Gyo-dong, Gyeongju	Hip bones	<i>Ascaris lumbricoides</i>	58.3 \pm 3.1 (55.0–62.5) \times 45.0 \pm 0.0 (45.0–45.0)	46.6
		<i>Trichuris trichiura</i>	49.7 \pm 0.5 (49.0–50.0) \times 23.8 \pm 0.6 (23.0–24.4)	32.8
	Sacrum	<i>Ascaris lumbricoides</i>	62.5 \pm 2.0 (60.0–65.0) \times 50.2 \pm 1.8 (48.0–52.5)	48.3
		<i>Trichuris trichiura</i>	50.6 \pm 0.6 (50.0–51.2) \times 25.5 \pm 0.5 (25.0–26.0)	62.1

Values are presented as average \pm standard variation (range).
EPG = eggs per gram.

life cycle of soil-transmitted parasites, this means that even Silla nobles might have ingested foods or contacted soils seriously contaminated by human feces or derivative materials. Indeed, our parasitological analyses of archaeological samples from a variety of chronological eras can move us even closer to the goal of elucidating the parasitological infection patterns spanning Korean history.

REFERENCES

1. Ferreira LF, Reinhard KJ, Araújo AJ. *Fundamentos da Paleoparasitologia*. Rio de Janeiro, Brazil: Editora Fiocruz; 2011.
2. Reinhard K, Araújo A. Archaeoparasitology. In: Pearsall DM, editor. *Encyclopedia of Archaeology*. San Diego, CA: Academic Press; 2008, 494-591.
3. Seo M, Araujo A, Reinhard K, Chai JY, Shin DH. Paleoparasitological studies on mummies of the Joseon Dynasty, Korea. *Korean J Parasitol* 2014;52(3):235-42.
[PUBMED](#) | [CROSSREF](#)
4. Bouchet F, Baffier D, Girard M, Morel P, Paicheler JC, David F. Paléoparasitologie en contexte pléistocène: premières observations à la Grande Grotte d'Arcy-sur-Cure (Yonne), France. *C R Acad Sci III* 1996;319(2):147-51.
5. Evans AC, Markus MB, Mason RJ, Steel R. Late stone-age coprolite reveals evidence of prehistoric parasitism. *S Afr Med J* 1996;86(3):274-5.
[PUBMED](#)
6. Gonçalves ML, Araújo A, Ferreira LF. Human intestinal parasites in the past: new findings and a review. *Mem Inst Oswaldo Cruz* 2003;98 Suppl 1:103-18.
[PUBMED](#) | [CROSSREF](#)
7. Seo M, Guk SM, Kim J, Chai JY, Bok GD, Park SS, et al. Paleoparasitological report on the stool from a medieval child mummy in Yangju, Korea. *J Parasitol* 2007;93(3):589-92.
[PUBMED](#) | [CROSSREF](#)
8. Seo M, Shin DH, Guk SM, Oh CS, Lee EJ, Shin MH, et al. *Gymnophalloides seoi* eggs from the stool of a 17th century female mummy found in Hadong, Republic of Korea. *J Parasitol* 2008;94(2):467-72.
[PUBMED](#) | [CROSSREF](#)
9. Seo M, Oh CS, Chai JY, Lee SJ, Park JB, Lee BH, et al. The influence of differential burial preservation on the recovery of parasite eggs in soil samples from Korean medieval tombs. *J Parasitol* 2010;96(2):366-70.
[PUBMED](#) | [CROSSREF](#)
10. Shin DH, Lim DS, Choi KJ, Oh CS, Kim MJ, Lee IS, et al. Scanning electron microscope study of ancient parasite eggs recovered from Korean mummies of the Joseon Dynasty. *J Parasitol* 2009;95(1):137-45.
[PUBMED](#) | [CROSSREF](#)
11. Shin DH, Oh CS, Lee SJ, Lee EJ, Yim SG, Kim MJ, et al. Ectopic paragonimiasis from 400 year old female mummy of Korea. *J Archaeol Sci* 2012;39(4):1103-10.
[CROSSREF](#)
12. Seo M, Chai JY, Kim MJ, Shim SY, Ki HC, Shin DH. Detection trend of helminth eggs in the strata soil samples from ancient historic places of Korea. *Korean J Parasitol* 2016;54(5):555-63.
[PUBMED](#) | [CROSSREF](#)
13. Lee WJ, Woo EJ, Oh CS, Yoo JA, Kim YS, Hong JH, et al. Bio-anthropological studies on human skeletons from the 6th century tomb of ancient Silla Kingdom in South Korea. *PLoS One* 2016;11(6):e0156632.
[PUBMED](#) | [CROSSREF](#)
14. Han ET, Guk SM, Kim JL, Jeong HJ, Kim SN, Chai JY. Detection of parasite eggs from archaeological excavations in the Republic of Korea. *Mem Inst Oswaldo Cruz* 2003;98 Suppl 1:123-6.
[PUBMED](#) | [CROSSREF](#)
15. Pike AW. Recovery of helminth eggs from archaeological excavations, and their possible usefulness in providing evidence for the purpose of an occupation. *Nature* 1968;219(5151):303-4.
[PUBMED](#) | [CROSSREF](#)
16. Reinhard K, Urban O. Diagnosing ancient diphyllobothriasis from Chinchorro mummies. *Mem Inst Oswaldo Cruz* 2003;98 Suppl 1:191-3.
[PUBMED](#) | [CROSSREF](#)
17. Van Cleave HJ, Ross JA. A method for reclaiming dried zoological specimens. *Science* 1947;105(2725):318.
[PUBMED](#) | [CROSSREF](#)

18. Seo M, Oh CS, Hong JH, Chai JY, Cha SC, Bang Y, et al. Estimation of parasite infection prevalence of Joseon people by paleoparasitological data updates from the ancient feces of pre-modern Korean mummies. *Anthropol Sci* 2017;125(1):9-14.
CROSSREF
19. Korea Centers for Disease Control and Prevention. *The Report on the 8th National Survey for Intestinal Parasite Infection in Korea*. Cheongwon, Korea: Korea Centers for Disease Control and Prevention; 2013.