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BRIEF COMMUNICATION

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Paleoparasitological Surveys for Detection of Helminth Eggs in Archaeological Sites of Jeolla-do and Jeju-do

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Abstract: A paleoparasitological survey to detect helminth eggs was performed in archaeological sites of Jeolla-do and Jeju-do, the Republic of Korea. Total 593 soil samples were collected in 12 sites of Jeolla-do and 5 sites of Jeju-do from April to November 2011, and examined by the methods of Pike and coworkers. A total of 4 helminth eggs, 2 eggs each for *Trichuris trichiura* and *Ascaris* sp., were found in soil samples from 1 site, in Hyangyang-ri, Jangheung-eup, Jangheung-gun, Jeollanam-do. The egg-recovery layer was presumed to represent a 19th century farm, which fact suggested the use of human manures. This is the third archaeological discovery of parasite eggs in Jeolla-do. Additionally, no helminth eggs in archaeological sites of Jeju-do is an interesting problem to be solved in the further investigations.

Key words: *Ascaris*, *Trichuris trichiura*, paleoparasitology, Jeolla-do, Jeju-do

Parasitological examination of human coprolites obtained from archaeological sites is a proved useful tool for reconstructing of parasitic infection patterns in historical populations [1]. Although paleoparasitological examination of coprolites from mummies or soil samples has revealed various helminth eggs in Korea, most of the ancient parasite-egg discovery sites to date are confined to Seoul and Gyonggi-do [2-4]. Helminth eggs were also discovered in archaeological sites of Chungcheong-do and Gyeongsang-do [5-9]. Especially, Shin et al. [5] detected helminth eggs in the moat ruins of the Royal Palace of Silla Dynasty in Gyeogju-si, Gyeongsangbuk-do.

In Jeolla-do, only 2 paleoparasitological surveys had been performed. The 7th century soil samplings from the Wanggung-ri site, Iksan, Jeollabuk-do, revealed the eggs of *Ascaris lumbricoides*, *Trichuris trichiura*, and *Clonorchis sinensis*. Also *A. lumbricoides* and *T. trichiura* eggs were discovered in soils from Shinchang-dong, Gwangju, dating to 2,000 years BP [10,11]. However, there have been no reports on paleoparasitological discovery in Jeju-do, the home of 63,093 people in 1,435 [12].

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Hence, the present study was investigated for the presence of parasite eggs in both Jeolla-do and Jeju-do. In addition, environmental factors such as pH, temperature, and soil natures had been considered to effect the preservation of eggs. Although the effect was not larger than that of temperature, the changes in pH of the samples exhibited a little effect on the destruction or viability of parasite eggs [13]. In this study, the pH of their soils were measured to know the relationship with the egg preservation.

The sampling sites were selected in consultation with archeologists, who had excavated ancient remains of Jeolla-do and Jeju-do from April to November 2011. Detailed information has been provided in Table 1 and Table 2, respectively. Except in the case of tombs, soil-strata samples were collected by driving sterilized conical tubes deep into the archaeological layers. Additionally, for comparative purposes, surface-soil samples were collected. For these 2 regions, 12 and 5 sites were selected, respectively, from which a total of 593 samples were collected, 507 from Jeolla-do and 86 from Jeju-do. After transportation to our laboratory, the soil samples were prepared for light microscopic observations as described in Pike et al. [14]. If a parasite egg was discovered, it was identified for its species, and its density was measured. Sample pH also was measured, according to the AOAC Official method for the mineral soil [15], by which air-dried soil samples are mixed with H_2O, and

Table 1. Sampling sites and their information of Jeolla-do

Area	Period	Characteristics	No. of samples
Ungcheon-dong, Yeosu, Jeollanam-do	Bronze age	Dolmen	22
Songha-dong, Nam-gu, Gwangju-si	Bronze age	Agricultural land	19
Hyangyang-ri, Jangheung-eup, Jangheung-gun, Jeollanam-do	Bronze age	Tombs, ditches, trap pit	64
Sin-ri, Gokseong-gun, Jeollanam-do	Iron age	Pits	28
Songhak-dong, Iksan-si, Jeollabuk-do	Proto-three kingdom period	Unknown	28
Hwabang-ri, Wolsan-myeon, Damyang-gun, Jeollanam-do	Period of three states	Rice paddy	93
Seongsan-ri, Haerong-myeon, Suncheon-si, Jeollanam-do	4-5C	Residential areas	27
Musu-ri, Yudeung-myeon, Sunchang-gun, Jeollanam-do	4-5C	Residential areas	1
Hoejin-ri, Dasi-myeon, Naju-si, Jeollanam-do	Koryo Dynasty	Residential area & kiln	52
Yonggang-ri, Jangpyeong-myeon, Jangheung-gun, Jeollanam-do	Koryo Dynasty	Tombs & residential areas	95
Taekchon, Samyeong-dong, Naju-si, Jeollanam-do	Koryo Dynasty	Farm	8
Hyangyang-ri, Jangheung-eup, Jangheung-gun, Jeollanam-do	Joseon Dynasty	LSMB tombs, ditches, farm	70
Total 12 sites			507

Table 2. Sampling sites and their information of Jeju-do

Area	Period	Characteristics	No. of samples
Samyang 2-dong, Jeju-si	BC 7C	Presumed to be a toilet	2
Yongdam 2-dong, Jeju-si	BC 3C-AD 4C	Residential area	23
Oedo 1-dong, Jeju-si	1-4C	Pits	1
Hwasun-ri, Seogwipo-si	0-1C	Pits and drains	42
Geumsung-ri, Aewol-ri, Jeju-si	14-16C	Cemetery	18
Total 5 sites			86

the potential difference relative to standard soils was determined. The detailed procedures were as follows: The air-dried soil with known pH was selected as the standard one; 10 g of the standard soil was transferred into a cup, and mixed with 10 ml diatilled water. After vortexing for 5 sec, the samples were laid for 30 min. The pH of the standard sample was measured, and that of the test soil was measured in case of a permissible error (<0.1). The test soil was air-dried for 1-4 days, and ground into small pieces (<2 mm in diameter). The test soil was mixed with soil-H_2O as the case of standard soil. The pH of the test soil was measured at 20-25˚C.

No parasite eggs were discovered in the surface soil samples, which ruled out any false positivity induced by accidental introduction of surface soil into the investigated layers. However, most of the Jeolla-do and Jeju-do sites yielded no parasite eggs. Only at Hyangyang-ri, Jangheung-eup, Jangheung-gun, Jeollanam-do, 2 *Trichuris* sp. (Fig. 1) and 2 *Ascaris* sp. eggs (Fig. 2) were detected. The estimated number of eggs contained in the samples was 20.0 per gram of soil for both *Trichuris* and *Ascaris*. Each of the Ascaris eggs were 66.0 µm long by 55.0 µm wide and 66.0 µm long by 52.8 wide; 1 *Trichuris* egg was 52.5 µm long by 24.0 µm wide, by which size it was easily identified as *T. trichiura*. The other *Trichuris* egg, which was broken, could not be measured. All 4 eggs were recovered from the layer corresponding to the farm of a 19th century. As already noted, no parasite eggs were recovered from other samples, not even from additional samples collected in the same region of Hyangyang-ri. As for the soil pH, the only positive site was Hyangyang-ri, where 6.71 was recorded. The other sites' pH ranged from 4.69 to 6.91.

From the surveyed areas, the parasite eggs were only recovered from a site of Hyangyang-ri, Jeollanam-do. This is counter-intuitive considering the widespread and intensive utilization of human excreta as fertilizer in the Honam region (Jeolla-do), the breadbasket of Korea. Furthermore, while the soils from lyme soil mixture barrier (LSMB) tombs revealed many parasite eggs in spite of complete decomposition of the inside mummies in other provinces, the eggs could not be found through the investigation of 4 LSMB tombs in Jeolla-do and 1 LSMB tomb in Jeju-do. This might suggest that the eggs had not been preserved for a long time in these areas, but more extensive investigations seem to be needed for a conclusive remark.

While it was known that the amount of ammonia was in-

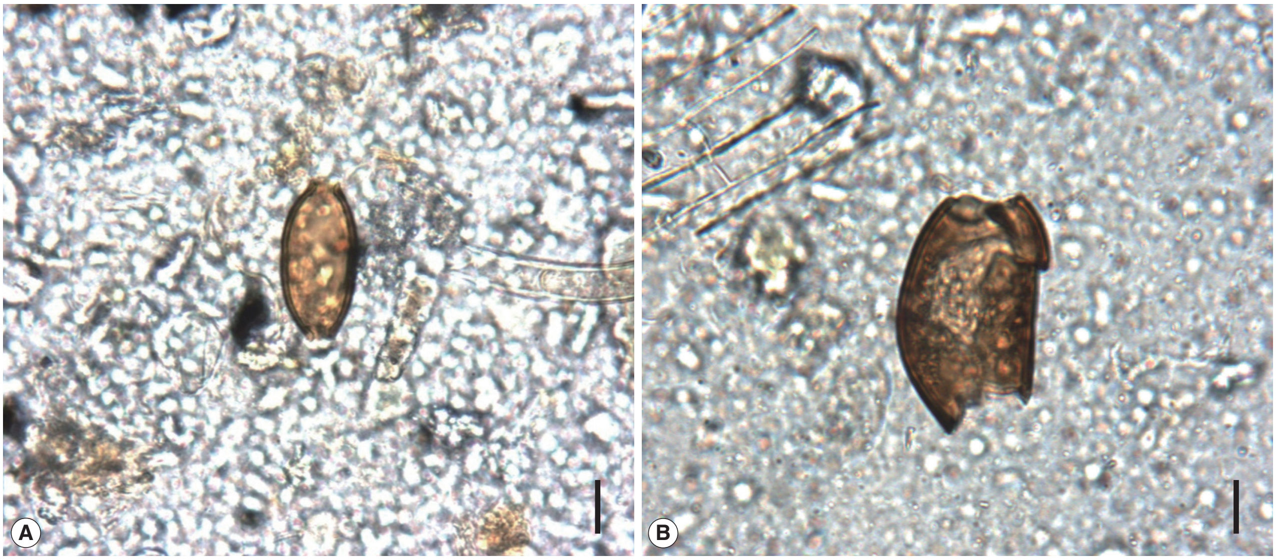

Fig. 1. Two *Tricuris trichiura* eggs (A, B) found in Hyangyang-ri sample. Bar = 20 µm.

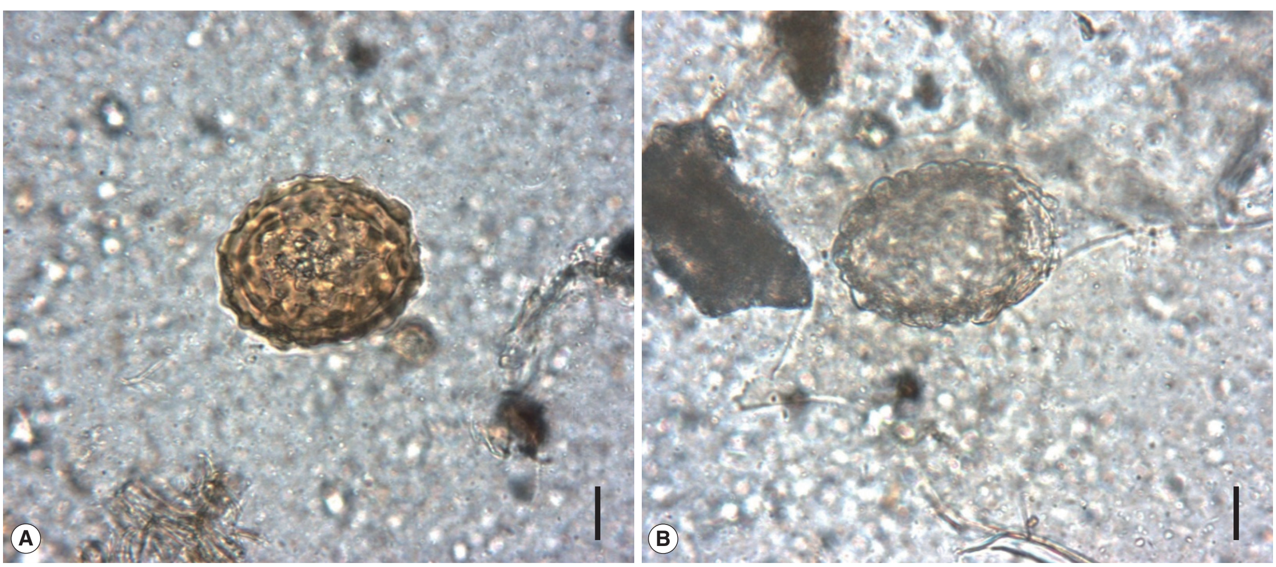

Fig. 2. Two *Ascaris* sp. eggs (A, B) found in the same sample. Bar = 20 µm.

versely correlated with the viability of parasite eggs [16], the predictors for long-term preservation of them had not been studied yet. In a previous study, there was a close correlation between the preservation of certain types of human remains and the presence of ancient parasite eggs in LSMB tombs, suggesting that such remains was a strong predictor of well-preserved ancient parasite eggs [4]. Another predictor is supposed to be the presence of water in the soil samples, as seen in the ancient moat ruins of Silla Dynasty [9]. The archaeological remains of Shinchang-dong, Gwangju, where the parasitic eggs had been discovered, were belonged to be the morass [11], and the large toilet of Wanggung-ri, Iksan-si, Jeollabuk-do, was connected to the drainage [10]. Decisively, the only positive samples of this study were collected from the farm connected with the drainage. It could be suggested that the moisture-laden environment helped the soils preserve parasite eggs for a long time.

However, the correlation between the soil pH and the preservation of eggs was not clearly observed due to the small number of positive samples. The soil pH of Hyangyang-ri, the only positive area, was 6.71, higher than that of Wanggung-ri, 4.68-5.24 [10]. According to the study of latrine soils in West Germany, no statistical relationship between the number of recovered eggs and soil pH was demonstrated [17]. Nevertheless, a conclusion that the soil pH is irrelevant to the egg preservation should be too hasty, and further studies are needed on more samples, especially on the egg-discovered sites in Korea.

In this study, the egg of *Trichuris* could be easily identified as that of *T. trichiura*. While the widths of *T. suis* eggs were mostly distributed between 28-30 µm, those of *T. trichiura* eggs ranged 25-26 µm, compatible with that of this study [18]. Moreover, the egg size of *T. vulpis* reached 90×44 µm in average [19]. However, with morphological characteristics only, it was impossible to know if the eggs were of human or pig origin in the cases of *Ascaris*. Nevertheless, the present eggs seemed to be of human origin in consideration that the eggs of *Trichuris* were that of *T. trichiura*. Regardless of their origin, these eggs seemed to be related to the agriculture. The archaeological remain where the parasite eggs were recovered was presumed to be the farm, and the presence of eggs might prove the use of human manures at that time. In fact, human manures were used as a solid-compost from the 15th century, and later mixed with ashes for the fertilizer on the paddy fields and dry fields [20]. Hence, further investigations on Jeolla-do should be concentrated into the farms to discover more ancient eggs along with extensive surveys on Jeju-do.

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