

## *Enterobius vermicularis* Eggs Discovered in Coprolites from a Medieval Korean Mummy

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**Abstract:** While the presence of pinworm eggs in archaeological samples has been reported by many researchers in the New World, those have been detected very scarcely in the Old World, especially in East Asian countries. In fact, many parasite species were recovered from the archeological remains in Korea, eggs of *Enterobius vermicularis* had not been found. Recently, a female mummy buried in the 17th century was discovered in the Joseon tomb from Dangjin-gun, Chungcheongnam-do, Korea. After rehydration process for 12 days, investigations were carried on the luminal surface of the colon. From them, 3 eggs of *E. vermicularis* were recovered. They were elliptical, transparent with a thin egg shell,  $50.3 \pm 5.2$   $\mu\text{m}$  (length) and  $28.2 \pm 3.9$   $\mu\text{m}$  (width) in size. This is the first discovery of *E. vermicularis* eggs in East Asia.

**Key words:** *Enterobius vermicularis*, mummy, egg, 17th century

Paleoparasitologists in Korea have examined ancient human samples from archeological fields in Korea, studying parasite infections prevalent in the past. Parasite eggs or larvae remained in mummies of Joseon Dynasty (1392-1910 AD) or in soil sediments from archeological sites became invaluable resources for studying ancient parasites that had infected Korean population. We have reported various ancient parasite species, including *Ascaris lumbricoides*, *Trichuris trichiura*, *Metagonimus yokogawai*, *Clonorchis sinensis*, *Paragonimus westermani*, *Gymnophaloides seoi*, *Strongyloides stercoralis*, and *Trichostrongylus* spp. [1-3]. However, even though some parasite eggs (e.g. pinworms and hookworms) were known to be one of the most common parasite species infecting Korean population, we still did not observe them in ancient samples.

In particular, the pinworm, *Enterobius vermicularis*, is a helminth infecting nearly a billion people worldwide in all socio-economic levels [4,5]. The parasite can be transmitted from host to host without stages in soil or intermediary hosts. In case of New World countries, the presence of pinworm eggs in ar-

cheological samples has been reported by many researchers. Briefly, *E. vermicularis* infection was proven among parasitological samples from ancient people in North America. The parasite eggs were found in a 10,000-year-old human coprolite from Utah, USA, one of the oldest human coprolites ever found and in the mummies' coprolites from several North American archaeological sites [6]. *E. vermicularis* infection was also identified in ancient Andean peoples. The pinworm eggs were observed in ancient coprolites from Chile, Peru, and Argentina [6].

However, interestingly enough, *E. vermicularis* has been detected very scarcely in the Old World [5,7]. Up to the present, there were only 2 reports of *E. vermicularis* eggs in Roman latrines [8] and in an Egyptian mummy [9]. Especially in case of East Asian countries, there were no reports on the presence of *E. vermicularis* in archeologically obtained samples. In this regard, the current case, a new Joseon mummy discovered in an archeological site of Korea, should be significant to concerned researchers in Korea. We found well-preserved *E. vermicularis* eggs, at this moment, showing the first-ever evidence for the preservation of ancient pinworm eggs in archeologically obtained samples from East Asian countries.

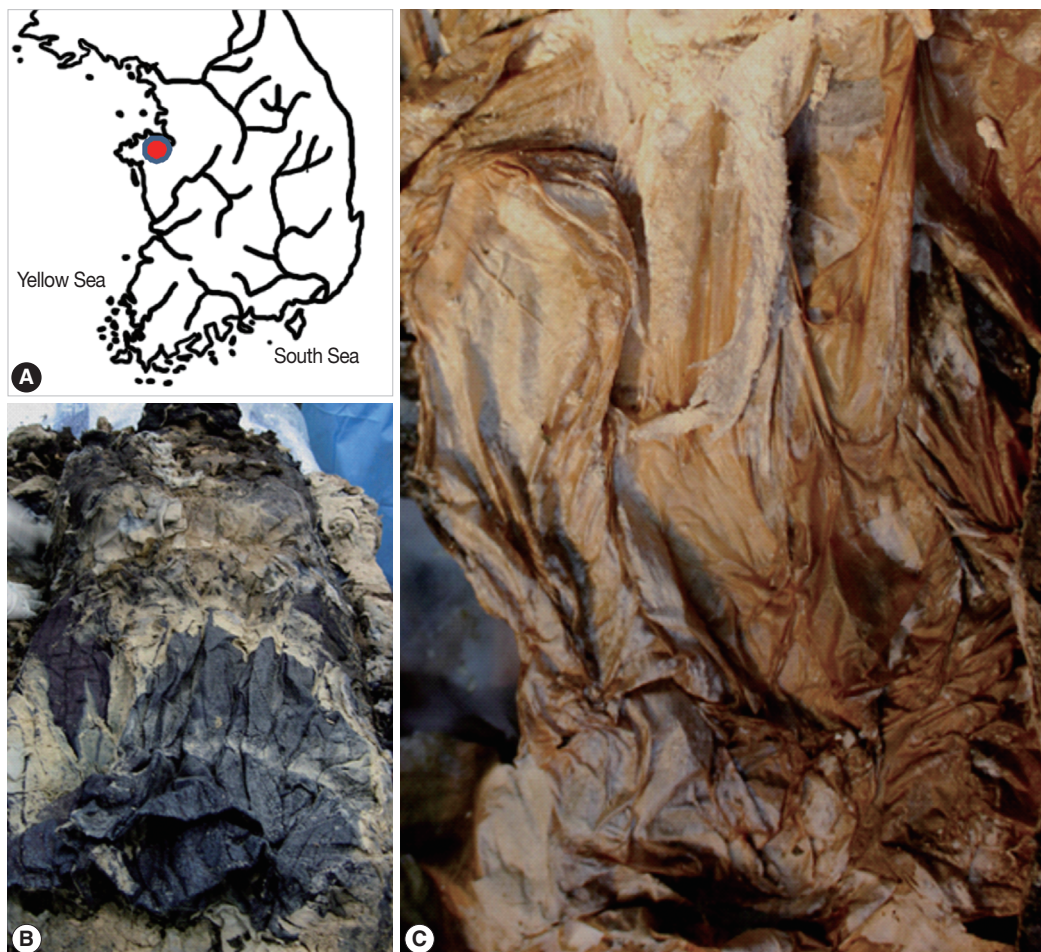
On August 2008, a female mummy was discovered in the Joseon tomb from Dangjin-gun, Chungcheongnam-do, Korea (Fig. 1A). Based on the tree-ring test, the tomb was confirmed to be constructed in 1630s AD [10]. After the mummy was mov-

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**Fig. 1.** (A) The location of Dangjin-gun (red dot), Chungcheongnam-do, Korea, where the female mummy was discovered. (B) The female mummy, presumably buried in 1630s AD. (C) The mummified intestines were dissected from the abdominal cavity.

ed to our laboratory, the researchers wearing sterilized gowns, gloves, head caps, and masks removed the clothes wrapped around the dead body (Fig. 1B). Anthropologists dissected the abdominal cavity in which mummified intestines were preserved very well (Fig. 1C). We collected parasitological samples from luminal surfaces of ascending, transverse, and descending colons.

Obtained samples were rehydrated in 0.5% trisodium phosphate solution for 12 days with occasional shaking [11]. They were then filtered through multiple-layered gauze, after which, were precipitated for a day. After the upper turbid layer was discarded, the precipitates were dissolved again in 0.5% trisodium phosphate solution. The solution was finally dropped onto slides for examination under a light microscope (BH-2, Olympus, Tokyo, Japan). The sizes of the parasite eggs were measured.

In the light microscopic observation, we observed 2 eggs of

*Paragonimus westermani*, 8 of *Ascaris lumbricoides*, and 3 of *Enterobius vermicularis*. As for *E. vermicularis* eggs, the average size was  $50.3 \pm 5.2 \mu\text{m}$  (length) by  $28.2 \pm 3.9 \mu\text{m}$  (width). *E. vermicularis* eggs showed typical characteristics; small, elliptical, and transparent with a thin eggshell (Fig. 2). The average size of *A. lumbricoides* eggs was  $66.0 \pm 5.7 \times 50.9 \pm 6.8 \mu\text{m}$ , and those of *P. westermani* were  $75.5 \pm 1.7 \times 48.3 \pm 1.8 \mu\text{m}$  in the state of operculum-missing.

Besides the common paleoparasitological interest on whether specific species of parasites infected people buried in ancient tombs, studies on *E. vermicularis* have special meaning to concerned researchers. Briefly, it is considered that the human-*E. vermicularis* relationship started in pre-hominid times, having evolved in Africa, and then dispersed to other continents by prehistoric human migrations [6,12,13]. Interestingly enough, many paleoparasitologists believed that ancient pinworms crossed the Bering Land Bridge with its human host during

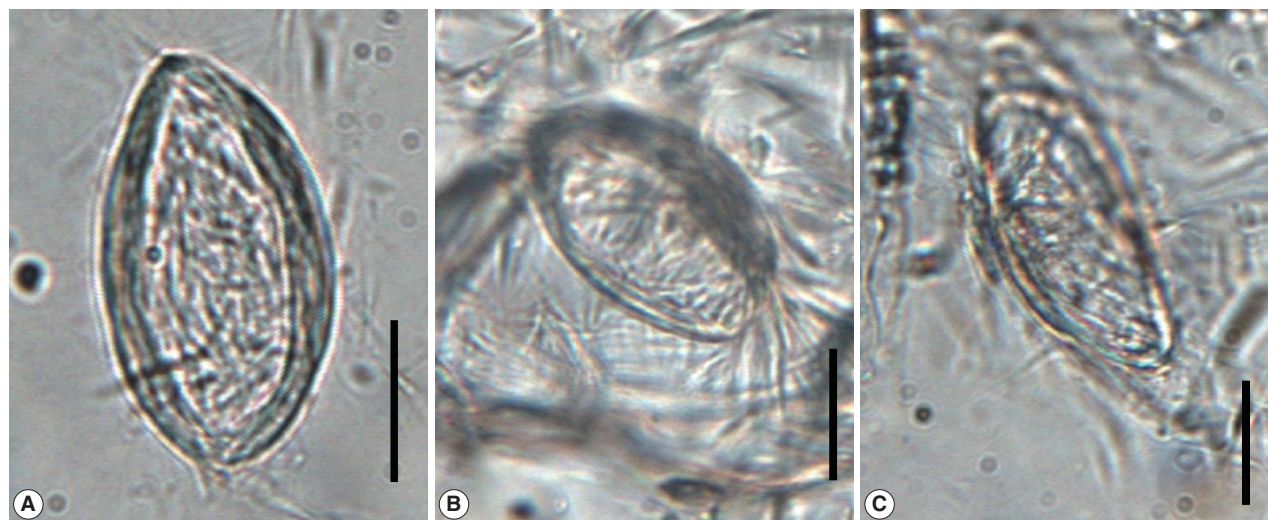


Fig. 2. Microscopic examination of 3 *Enterobius* eggs recovered from the mummy. Bar = 20  $\mu$ m.

their first migration to the Americas even if transpacific routes have also been postulated by some paleoparasitologists [6].

However, though there were many reports on the presence of *E. vermicularis* in the samples from archeological fields, observation of parasite eggs was very rarely reported from East Asian countries in spite of its endemicity. Of course, the preservation status varies in different species of parasite eggs. Relatively poorer preservation status of *E. vermicularis* eggs was well noted for archeologically obtained fecal remains [6,14]. However, considering that *E. vermicularis* infection reached very high prevalence as shown in some prehistoric coprolites containing ancient eggs [5,15], the absence of reports on the presence of *E. vermicularis* eggs from East Asian countries looks very strange.

For the past several years, though we have tried to locate the ancient *E. vermicularis* eggs in many samples from archeological fields, we could not find any. In this regard, the current report on the ancient *E. vermicularis* eggs from several-hundred-year old Joseon mummy could be a contribution to future studies over the issue. In addition, we showed the possibility that ancient *E. vermicularis* eggs could also be remained in the samples from archeological sites in East Asia for the first time. Though we are not sure why ancient *E. vermicularis* eggs in Old World countries were not preserved as perfectly as seen in New World counterparts, more detailed and cautious examinations on the archeological samples could provide invaluable information on the migration of ancient pinworms crossing the Bering Land Bridge with its human host.

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## REFERENCES

1. Seo M, Guk SM, Kim J, Chai JY, Bok GD, Park SS, Oh CS, Kim MJ, Yil YS, Shin MH, Kang IU, Shin DH. Paleoparasitological report on the stool from a Medieval child mummy in Yangju, Korea. *J Parasitol* 2007; 93: 589-592.
2. Seo M, Shin DH, Guk SM, Oh CS, Lee EJ, Shin MH, Kim MJ, Lee SD, Kim YS, Yi YS, Spigelman M, Chai JY. *Gymnophalloides seoi* eggs from the stool of a 17th century female mummy found in Hadong, Republic of Korea. *J Parasitol* 2008; 94: 467-472.
3. Shin DH, Chai JY, Park EA, Lee W, Lee H, Lee JS, Choi YM, Koh BJ, Park JB, Oh CS, Bok GD, Kim WL, Lee E, Lee EJ, Seo M. Finding ancient parasite larvae in a sample from a male living in late 17th century Korea. *J Parasitol* 2009; 95: 768-771.
4. Lukes J, Horák A, Scholz T. Helminth genome projects: All or nothing. *Trends Parasitol* 2005; 21: 265-266.
5. Iñiguez AM, Reinhard KJ, Araújo A, Ferreira LF, Vicente AC. *Enterobius vermicularis*: Ancient DNA from North and South American human coprolites. *Mem Inst Oswaldo Cruz* 2003; 98: 67-69.
6. Gonçalves ML, Araújo A, Ferreira LF. Human intestinal parasites in the past: New findings and a review. *Mem Inst Oswaldo Cruz* 2003; 98: 103-118.
7. Bouchet F, Guidon N, Dittmar K, Harter S, Ferreira LF, Chaves SM, Reinhard K, Araújo A. Parasite remains in archaeological sites. *Mem Inst Oswaldo Cruz* 2003; 98: 47-52.
8. Herrmann B. Parasitologisch-epidemiologische auswertungen mittelalterlicher kloaken. *Z Archaol Mittelalters* 1985; 13: 131-

- 161.
9. Horne PDJ. First evidence of enterobiasis in ancient Egypt. *J Parasitol* 2002; 88: 1019-1021.
  10. Chungnam Institute of History and Culture, Dangjin Uduri Yujok (II). Gongju. 2010, p 553-562.
  11. Van Cleave HJ, Ross JA. A method for reclaiming dried zoological specimens. *Science* 1947; 105: 318.
  12. Hugot JP, Reinhard KJ, Gardner SL, Morand S. Human enterobiasis in evolution: Origin, specificity and transmission. *Parasite* 1999; 6: 201-208.
  13. Iñiguez AM, Reinhard K, Carvalho Gonçalves ML, Ferreira LE, Araújo A, Paulo Vicente AC. SL1 RNA gene recovery from *Enterobius vermicularis* ancient DNA in pre-Columbian human coprolites. *Int J Parasitol* 2006; 36: 1419-1425.
  14. Reinhard KJ, Hevly RH, Anderson GA. Helminth remains from prehistoric Indian coprolites on the Colorado Plateau. *J Parasitol* 1987; 73: 630-639.
  15. Reinhard KJ. Mummy studies and archaeoparasitology. In Cockburn A, Cockburn E, Reyman TA, eds, *Mummies, Disease and Ancient Cultures*. Cambridge, UK. Cambridge University Press. 1998, pp 377-380.