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**A Case of Ectopic Paragonimiasis in a 17th Century Korean Mummy**

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**ABSTRACT:** Archaeoparasitological studies on fossilized feces obtained from Joseon Dynasty (1392–1910 CE) mummies have provided invaluable data on the patterns of parasitic infection in pre-modern Korean societies. In our recent radiological investigation of a 17th century Joseon mummy discovered in Cheongdo (South Korea), we located a liver mass just below the diaphragm. Anatomical dissection confirmed the presence of a mass of unknown etiology. A subsequent parasitological examination of a sample of the mass revealed a large number of ancient *Paragonimus* sp. eggs, making the current report the first archaeoparasitological case of liver abscess caused by ectopic paragonimiasis.

Remarkably well-preserved mummies have been discovered in tombs dating to the Joseon Dynasty (1392–1910 CE) of Korea. The researchers have speculated that the unique mummification process in Korea might be caused by very complicated physicochemical reactions inside the grave. The typical Joseon tomb's construction (including a coffin-encompassing lime-soil wall and/or a charcoal-lined grave, etc.) might also have contributed to the mummification (Kim et al., 2008; Oh and Shin, 2014). However, the exact mechanism remains a mystery.

In any case, what is undeniably true is Joseon mummies' indispensability to studies that have shed much light on the health and disease statuses of Joseon-period populations. Indeed, through morphological and molecular analyses of mummy samples, we have acquired invaluable information on ancient pathology that could not have been gained by any other means.

Ancient-parasite infections have been identified in samples obtained from mummies discovered all over the world. South American mummies, for example, have shown evidence of *Entamoeba* sp., *Trichuris* sp., *Echinostoma* sp., *Trypanosoma* sp., and *Leishmania* sp. infections (Horne and Kawasaki, 1984; Guhl et al., 1999; Sianto et al., 2005; Dageförde et al., 2014; Panzer et al., 2014; Novo et al., 2015; Reinhard and Araújo, 2016). Chinese mummies have yielded indications of *Clonorchis sinensis*, *Trich-*

*uris* sp., *Ascaris* sp., and *Enterobius vermicularis* infections (Wei et al., 1981; Li, 1984; Liu et al., 2007; Yeh and Mitchell, 2016), while an Iranian mummy sample revealed a *Taenia* sp. infection (Nezamabadi et al., 2013).

Likewise, our previous investigations of Korean mummies have identified various parasite species, including *Ascaris* sp., *Trichuris trichiura*, *C. sinensis*, *Paragonimus westermani*, *Metagonimus yokogawai*, *Gymnophaloides seoi*, *E. vermicularis*, and *Taenia* sp. (Oh et al., 2010a, 2010b, 2015; Shin et al., 2011, 2012a, 2012b, 2013; Seo et al., 2014a, 2014b). As alluded to above, Joseon mummies are now of central importance to archaeoparasitologists seeking to uncover the parasitic infection statuses of pre-modern Korean societies. Nonetheless, additional and more detailed research is still required in order to fully understand the reality of parasitism in Joseon society. The new parasitological report provided herein is perhaps a step in that direction.

On October 2014, a 17th century Joseon tomb was found in Cheongdo, a southeastern county in South Korea. In this tomb, together with well-preserved clothes, a mummy was discovered (Fig. 1). The specimen's preservation status was excellent. The



FIGURE 1. Cheongdo mummy.

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FIGURE 2. Radiological image of Cheongdo mummy. Liver mass (indicated by arrow) could be observed below the right sided diaphragm. Lv, liver.

external-genitalia morphology revealed an individual of the male sex. According to an archaeological report, the male (named Jing Lee) was born in 1580 and died on 6 November 1642 (aged 63). Under the auspices of his clan members (Kosung Lee), the mummy was moved to our lab for additional examinations. This study was exempted from Institutional Review Board (IRB) review by Seoul National University Hospital (IRB No. 2013-004).

Whole-body computed tomography (CT) scanning was performed using 64-MDCT scanner (VCT, GE Healthcare, Waukesha, Wisconsin) at Dankook University Hospital, South Korea. The scans were obtained in 0.625 mm thickness. Images were reviewed under various settings in multi-planar (axial, sagittal, and coronal images) formats using software on a workstation (Advantage Windows 4.3, GE Healthcare).

On the CT images, the internal organs of the Cheongdo mummy were shrunken to the dorsal side, doubtless due to the long-term effect of gravitational force. There were very few signs suggestive of specific pathological changes on the reformatted CT planes, except for a liver mass (22.75 mm × 23.06 mm) detected just below the right side of the diaphragm (Fig. 2). A faint

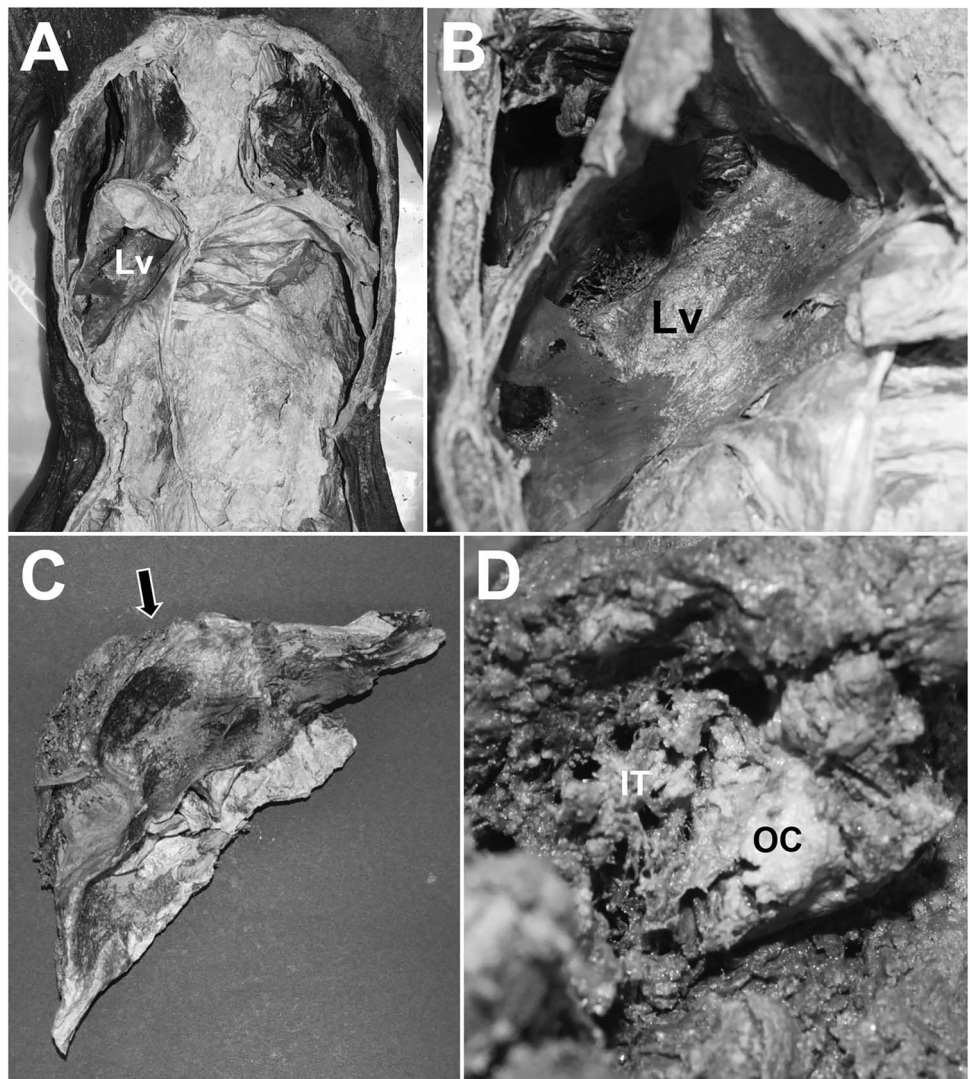


FIGURE 3. Dissection of the mummy. (A) Liver (Lv) is exposed by dissection. (B) Exposed mummified liver seen from below. (C) Mummy liver removed. Arrow indicates the portion where liver mass is situated. (D) Bisected liver mass. Outer-capsular (OC) and inner-trabecular (IT) parts could be differentiated.

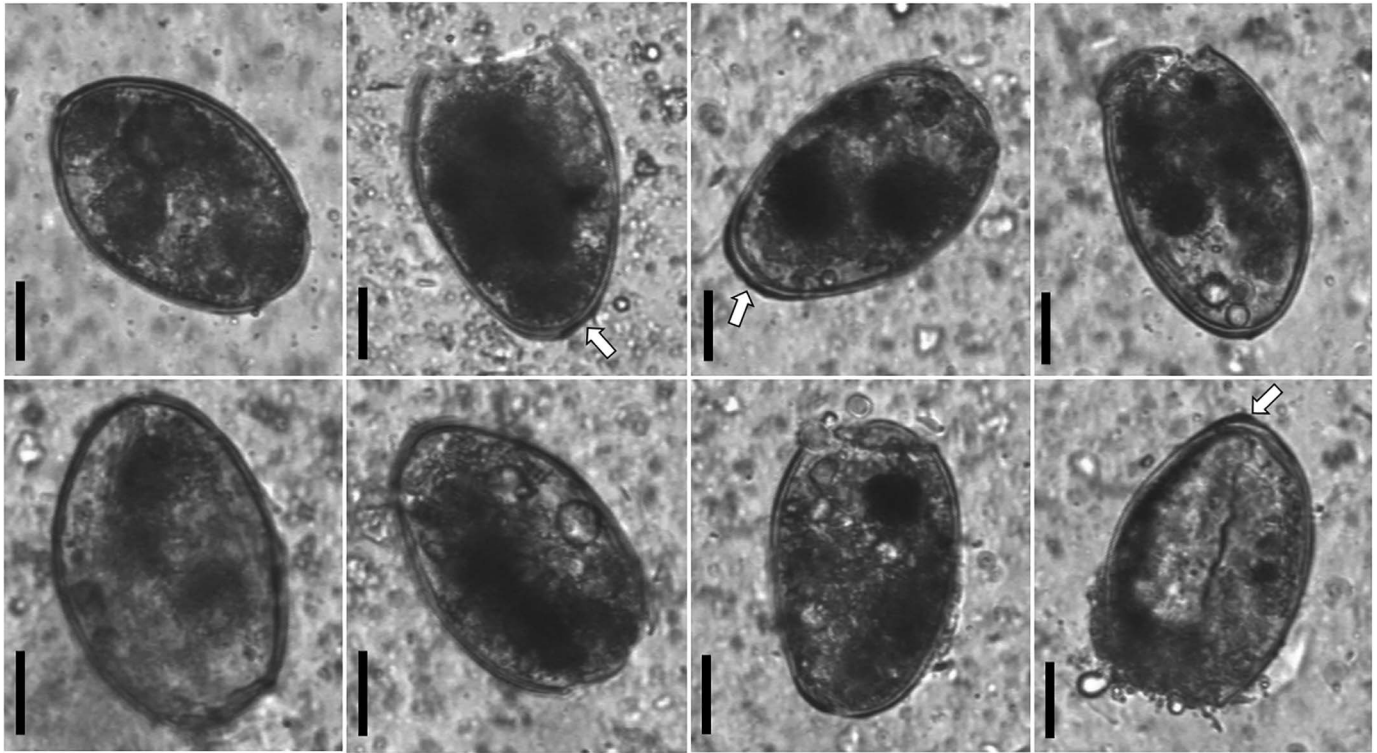


FIGURE 4. Ancient *Paragonimus* eggs discovered from the inner-trabecular material of liver mass. Arrows indicate abopercular thickenings. Scale bars in each egg plate = 20  $\mu\text{m}$ .

radiolucent pattern could be observed in the central area of the round radiopaque mass.

The nature of the liver mass could be identified by autopsy. An incision was made in the anterior part of the torso. As observed in the CT images, the mass was located in the liver just below the diaphragm. The liver was excised with careful removal of the perimeter. After observing the outward appearance of liver, we autopsied the left lobe until the mass was exposed. Upon bisection, the outer-capsular and inner-trabecular segments were easily distinguishable (Fig. 3). The inner-trabecular segment was thought to be the radiolucent area that had been noted on the CT images. We then sampled it for parasitological examination.

The obtained liver mass sample (1.3 g) was chopped into fine granules preparatory to rehydration in 0.5% trisodium phosphate solution. Subsequently the in-solution sample was shaken occasionally at room temperature for 1 wk, after which it was filtered through multilayered gauze. Following 1-day precipitation at room temperature, the upper turbid solution was discarded. The precipitates were dissolved again in 0.5% trisodium phosphate solution (final volume: 20 ml). The final solution was dropped onto slides for light microscope examination (Olympus, Tokyo, Japan). A total of 0.2 ml of dissolved solution was examined, 20  $\mu\text{l}$  on each slide, 10 times. We measured the size of the parasite eggs. The number of eggs per gram (EPG) was also estimated by the equation (20 ml/1.3 g  $\times$  the number of observed eggs/0.2 ml).

The microscopic examination revealed a number of *Paragonimus* sp. eggs. The estimated EPG was 307.7 (Fig. 4). The observed eggs were broadly ovoid, golden-brown in color, and

measured 84.0  $\mu\text{m}$  (length) by 51.5  $\mu\text{m}$  (width). This size well matched the commonly accepted size of *P. westermani* (70.0–100.0  $\mu\text{m}$   $\times$  (38.8–55.0  $\mu\text{m}$ ) (Miyazaki, 1991). Most of the eggs also exhibited an uneven thickness of the non-operculated end (i.e., abopercular thickening), which is a definitive diagnostic sign of *P. westermani*. We were able to rule out *Echinostoma* or *Fasciola hepatica*, as their opercula are indistinct and very small in comparison with that of *P. westermani* (Miyazaki, 1991).

*Paragonimus* sp. remains endemic in many countries; as many as 293.8 million people worldwide are still at risk of infection (Iwagami et al., 2000; Keiser and Utzinger, 2005; Liu et al., 2008; Shin et al., 2012b). Our archaeoparasitological studies on Joseon mummy coprolites have indicated a probable *P. westermani* infection prevalence rate as high as 27.8% in 16th–18th century Korea (Seo et al., 2014b). The estimate contrasts starkly with the 2012 National Survey Data (0.0%) for the same country (Korea Association of Health Promotion, 2012).

As a possible explanatory model for the high paragonimiasis prevalence in Korean history, scholars have identified the Joseon-cultural practice of ingesting raw freshwater crustaceans (the secondary intermediate host of *P. westermani*) either as a seasonal delicacy (raw crabs spiced with soybean sauces) or as a medication for measles (crayfish juice) (Yun, 1960; Cho et al., 1997; Sohn et al., 2009; Shin et al., 2012b). In this regard, the mummified Joseon male who had suffered from *P. westermani* infection presumably consumed raw or undercooked freshwater crustaceans in his lifetime (Shin et al., 2012b).

*Paragonimus* spp. can migrate to extra-pulmonary organs (ectopic paragonimiasis) including the brain, muscle, omentum,

liver, and others (Kim et al., 2004; Liu et al., 2008; Shin et al., 2012b). In addition to the current Cheongdo case, several years ago, we reported a finding of ancient *P. westermani* eggs in the liver parenchyme of a 17th century Joseon female mummy (Shin et al., 2012b). We speculate that the repeated observation of hepatic paragonimiasis in Korean mummy cases is related to the strong-infectivity-enhancing high prevalence of *P. westermani* infection among the Joseon people.

Our current report on the discovery of *Paragonimus* sp. eggs in a liver mass perhaps will be significant to concerned researchers. The total number of modern hepatic paragonimiasis cases is still very low. Liver masses induced by hepatic paragonimiasis are much less common, even in clinical medicine (Kim et al., 2004; Cheng et al., 2010; Cao and Qiu, 2012; Li et al., 2012a, 2012b; Shin et al., 2012b; Lu et al., 2013). This paper is, to the best of our knowledge, the first-ever report on ancient liver abscess caused by ectopic paragonimiasis.

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