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Case Study

Parasitism of the Zweeloo Woman: Dicrocoeliasis evidenced in a Roman period bog mummy

Nicole Searcey[a], Karl J. Reinhard[b,*], Eduard Egarter-Vigl[c], Frank Maixner[d], Dario Piombino-Mascali[d], Albert R. Zink[d], Wijnand van der Sanden[e], Scott L. Gardner[f], Raffaella Bianucci[g]

[a] Department of Biological Sciences, 348 Manter Hall, University of Nebraska – Lincoln, Lincoln NE 68588, United States
[b] School of Natural Resources, Hardin Hall 719, University of Nebraska – Lincoln, Lincoln, NE 68583-0987, United States
[c] Department of Pathology, General and Regional Hospital, Via L. Bohler 5, 39100 Bolzano, Italy
[d] EURAC – Institute for Mummies and the Iceman, Viale Druso 1, 39100 Bolzano, Italy
[e] Province of Drenthe, Westerbrink 1, 9405 BJ, Assen, The Netherlands
[f] Harold W. Manter Laboratory of Parasitology, W-529 Nebraska Hall, University of Nebraska State Museum and School of Biological Sciences, University of Nebraska – Lincoln, Lincoln, NE 68588-0514, United States
[g] Laboratory of Physical Anthropology, Department of Public Health and Pediatric Sciences, University of Turin, Corso G. Galilei 22, 10126 Turin, Italy

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ABSTRACT

We undertook the analysis of Zweeloo Woman, a bog mummy from the Netherlands, to assess her parasitic state. Evidence of infection came from two areas: (1) liver paraffin sections and (2) microfossils washed from an intestinal section. Although the liver had shrunken considerably, objects consistent with operculated trematode eggs were found. After evaluating the range of trematode species that produce eggs in liver tissue, we arrived at the diagnosis of *Dicrocoelium dendriticum*. Although only 0.1 ml of sediment was recovered from an intestinal section, eggs of *Ascaris lumbricoides* and *Trichuris trichiura* were also identified. No eggs of *D. dendriticum* were revealed by the intestinal wash although they were observed in the liver. The lancet fluke, *D. dendriticum*, is a zoonosis that usually infects ruminants such as cattle. Eggs of *D. dendriticum* may be found in human coprolites if infected cow liver, for example, was eaten. This is false parasitism. Since eggs of *D. dendriticum* were found in the liver of Zweeloo Woman, we are assured this was a true infection. This find is especially significant because it is the oldest known, patent infection of *D. dendriticum* in humans.

1. Introduction

Since mummies retain the combination of intestinal contents, soft tissue and hard tissue, they have more to reveal about parasitic infection and disease than any other class of archaeological remains. On December 5th, 1951, two peat-cutters found the mummified body of a woman in a bog. The location was known as the "Damsel's Bog", northwest of the villages of Aalden and Zweeloo (Province of Drenthe, the Netherlands). She was named "Zweeloo Woman" after the municipality in which she was found (van Zeist, 1952) (Fig. 1).

The Zweeloo Woman was an adult between 35 and 50 years of age at time of death, as assessed by the examination of bones and teeth (Bianucci et al., 2012). Radiocarbon dating performed on both skeletal and skin tissue suggested that Zweeloo Woman lived during the Roman period (average of two radiocarbon dates: 1861 ± 35 BP, calibrated 2σ: 78–233 cal AD) (van der Sanden, 1990). When discovered, she was unclothed and lying on her ventral side with her limbs oriented in a somewhat fetal position. Her remains consist of a nearly complete skeleton, internal organs, and skin.

Zweeloo Woman's place of interment lies within the boundaries of the village municipality (Marke) of Aalden, which dates back to the Middle Ages and probably long before. The eastern boundary is formed by the brook valley of the "Aalder Stroom". The Roman period settlement in which she may have lived has not been discovered. Most likely this settlement is still hidden under the plaggen soil "Aalder Esch", situated to the south of Aalden where an Early Medieval cemetery was excavated. The distance between the bog in which Zweeloo Woman was discovered and her assumed settlement is about 2.5–3 km.

Zweeloo Woman's intestines and other organs were preserved by anaerobic conditions in combination with natural tannic acid in the bog. In bog bodies, liver and kidneys are generally not as well-preserved as skin. These two visceral organs are commonly

* Corresponding author. Tel.: +1 402 875 2863.
E-mail address: kreinhard1@mac.com (K.J. Reinhard).

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Fig. 1. The location of Zweeloo in the Province of Drenthe, the Netherlands. Courtesy Groningen University, Groningen Institute of Archaeology.

reduced in size or are unrecognizable due to bog pressure. The lungs and intestinal walls (excluding epithelial lining) are the most commonly preserved and recognizable internal organs (Aufderheide, 2003). Fortunately, Zweeloo Woman's intestine, kidneys and liver were preserved.

2. Materials and methods

The intestine, liver and kidneys were identified by anatomical association and general morphology. Small tissue biopsies (0.5 cm × 0.5 cm) were taken from the liver and kidney. They were analyzed following the methods described in Mekota and Vermehren (2005). After rehydration in Solution III for 48 h, samples were fixed for 24 h in 4% formaldehyde, dehydrated, and finally embedded in paraffin blocks, which were cut on a microtome in 3 μm thick sections (Leica, RM2245). These were histochemically counterstained with either hematoxylin and eosin stain (H&E) or Gram stain (Mulisch and Welsch, 2010).

An intestine section, approximately three centimeters long, was studied. No visible coprolites or other contents were observed in the section. Therefore, we devised a method, not before published, to recover botanical microfossils and parasites eggs. The section was placed in a gridded (1 cm^2) Petri plate and rehydrated using an aqueous solution of 0.5% trisodium phosphate. After treatment, the sample increased approximately 50% in size and regained the appearance and resilience of a fresh intestine section.

The exterior of the rehydrated intestine was washed for microscopic remains. The rehydration fluid from the Petri plate and the wash fluid were centrifuged in a 50 ml centrifuge tube to concentrate potential microscopic remains. This was labeled "exterior wash" and served as a control sample. Then the section was split along the longest dimension and the section was opened. The interior of the section was washed with a jet of distilled water into a clean beaker and gently scraped with a small lab spatula. The fluid from the interior wash was screened through a 250 μm mesh screen to trap macroscopic remains. The fluid that passed through the screen was captured in a 600 ml beaker and was concentrated by centrifugation. To loosen and recover as many microfossils as possible, the open intestine was sonicated for 30 s in a 50 ml centrifuge tube. The intestine was removed from the tube and the microresidues were concentrated by centrifugation. The two tubes of internal microresidues were combined together and labeled "interior wash".

Kumm et al. (2010) applied a newer method of quantifying parasite data based on earlier palynology methods (Maher, 1981; Reinhard et al., 2006). This is the "microfossil concentration method" that allows one to calculate the approximate number of microfossils, including parasites, per unit measure of coprolites by adding known numbers of exotic microfossils. This method can be applied to any other microresidues in archaeological samples. When the microresidues were screened into beakers, we added one tablet of *Lycopodium* (batch 212761, University of Lund, Sweden)