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Research article

Intestinal parasites from the 2nd–5th century AD latrine in the Roman Baths at Sagalassos (Turkey)

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ABSTRACT

The aim of this research was to determine the species of intestinal parasite present in a Roman Imperial period population in Asia Minor, and to use this information to improve our understanding of health in the eastern Mediterranean region in Roman times. We analyzed five samples from the latrines of the Roman bath complex at Sagalassos, Turkey. Fecal biomarker analysis using 5β-stanols has indicated the feces were of human origin. The eggs of roundworm (*Ascaris*) were identified in all five samples using microscopy, and the cysts of the protozoan *Giardia duodenalis* (which causes dysentery) were identified multiple times in one sample using ELISA. The positive *G. duodenalis* result at Sagalassos is particularly important as it represents the earliest reliable evidence for this parasite in the Old World (i.e. outside the Americas). As both these species of parasite are spread through the contamination of food and water by fecal material, their presence implies that Roman sanitation technologies such as latrines and public baths did not break the cycle of reinfection in this population. We then discuss the evidence for roundworm in the writings of the Roman physician Galen, who came from Pergamon, another town in western Asia Minor.

1. Introduction

Sagalassos in southwestern Turkey was the most important urban settlement in the region of Pisidia during the Roman Imperial period (Fig. 1). It was incorporated in the Roman Empire in 25 BC and remained prosperous into late antiquity. Its high altitude (1400–1600 m above sea level), fertile territory, access to fresh and salt water, and location along one of the key roads north from the Mediterranean harbors of Pamphylia all contributed to its establishment as a regional economic center based around the local ceramic industry (Sagalassos Red Slip Ware) and the production of cash crops such as grains and olives (Fuller et al., 2012; Poblome, 2015).

While a good number of Roman period sites have been analyzed for intestinal parasites, some parts of the Roman Empire have had no analysis whatsoever (Mitchell, 2017). These include central and western parts of North Africa (Libya, Tunisia, Morocco), the Iberian Peninsula (Spain and Portugal), the Mediterranean islands, and Asia Minor (Turkey). Indeed, there has been no parasite analysis of any archaeological sites from Turkey published, from any time period in history. Given the absence of data from Turkey, Sagalassos is an important site for understanding intestinal health in Roman Asia Minor.

The aim of this study is to determine the types of intestinal parasites that infected those people using the latrines at the Roman baths at Sagalassos in order to see how this region compares with other parts of the Roman Empire at that time. This will not only provide insight into the health of the inhabitants of Sagalassos, but also allow us to better understand their diet, cooking habits, level of sanitation, and agricultural practices. One of the key physicians of the classical world was Galen of Pergamon (c.130–c.210 AD), and Pergamon was a town which was also located in western Asia Minor. We will use the evidence for parasites in Galen's medical texts to understand what people in the Roman period thought of these diseases.

2. Materials

During excavation of the vaulted rooms supporting the western

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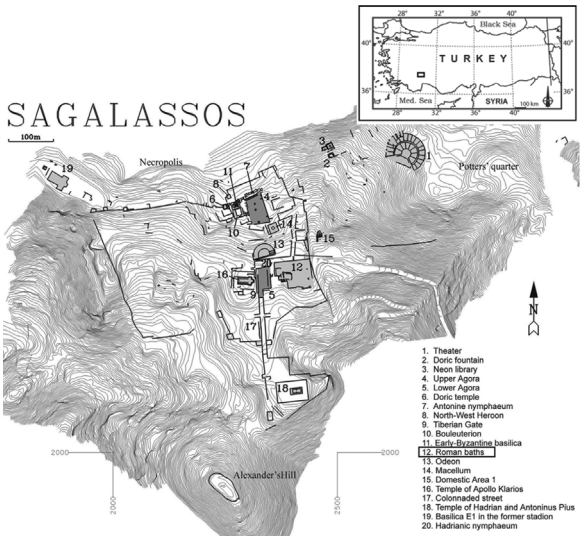

Fig. 1. Map showing location of Sagalassos in Asia Minor, and location of the Roman Baths (12) within the city.

Fig. 2. Photograph of the latrines (Room 4) at Sagalassos.

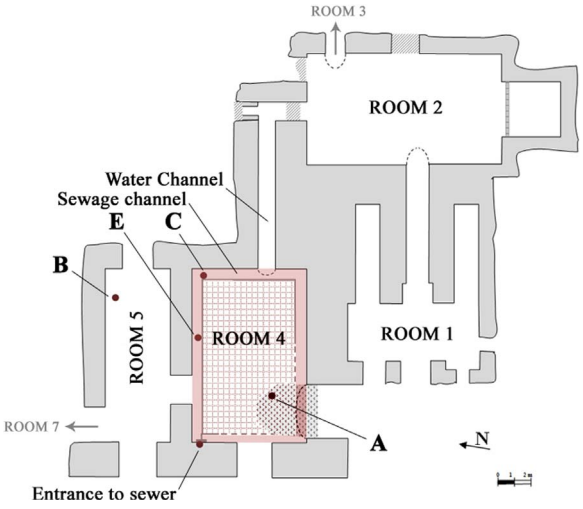

Fig. 3. Excavation plan of the latrine (Room 4) and adjacent rooms under the Roman Baths, showing sampling locations. The samples for which the sterol analysis indicates human feces originated from the sewage channel at sampling locations C and E.

parts of the imperial bath complex, a communal latrine was identified (Fig. 2). The actual toilet infrastructure had been removed in antiquity, but sufficient traces were left to positively identify Room 4 as a latrine. These include the holes in the mortared brick walls for affixing toilet seats, and the partially preserved sewage channels under where the toilet seats would have been. The sewage channels collected the excrement and drained it into the sewer opening in the north-west corner of the room. Water was recycled from the baths on the level above and entered the room through a channel in the eastern wall (Fig. 3). This washed the feces from under each seat along the sewage channels. In late antiquity the toilets seats were removed from the latrine and the room was used to collect urban refuse, and for the composting of animal manure (Baeten et al., 2012).

At the time of excavation multiple sediment samples were taken from Room 4 (samples A–E). The samples were analyzed at the University of Leuven (Belgium) using gas chromatography mass spectrometry (GCMS), atomic absorption spectroscopy (AAS) and microscopy to study fecal biomarkers, calcium concentration, and presence of plant macrofossils, pollen, and fungal spores. Fecal biomarkers can differentiate fecal material from different animal species. If the fecal material is omnivore (e.g. human) in origin then coprostanol and epicoprostanol will dominate, while in feces of herbivore origin 5β-stigmastanol and 5β-epistigmastanol will dominate (Linseele et al., 2013; Shillito et al., 2011). For the Sagalassos latrine the fecal biomarker results showed that the sediment above ground level in the vaulted room (sample A) represented decomposed animal dung from herbivores, with coprostanol: 5β-stigmastanol ratios around 0.05. In contrast, sediment in the sewage channels (samples C and E) represent decomposed omnivore feces with coprostanol: 5β-stigmastanol ratios within the range 1.0–3.45, compatible with a human origin (Baeten et al., 2012). Calibrated AMS radiocarbon dating of charcoal fragments from four samples suggests the fecal material dates from the 2nd–5th centuries AD (Baeten et al., 2012). Five samples with fecal biomarkers indicating human feces, and four samples with biomarkers indicating herbivore feces, were then sent to Cambridge for parasite analysis.

3. Methods

In order to analyze the samples for intestinal parasites, 0.2 g of dry latrine sediment was suspended in 5 ml 0.5% aqueous solution of trisodium phosphate until disaggregated. This suspension was passed through a series of stacked micro-sieves of mesh size 300 μm, 160 μm, and 20 μm. Most species of intestinal helminth that infect humans in this region produce eggs with dimensions between 30 μm and 150 μm, so the parasite eggs within the samples would be trapped on the mesh of the 20 μm sieve (Bouchet et al., 2003). After washing this material from the 20 μm sieve the resulting suspension was centrifuged to concentrate the sample, the supernatant discarded, and glycerol added before mounting on slides. Slides were viewed under a light microscope at 400× magnification to identify and quantify the parasite eggs present. Eggs were identified by shape, size, color, and special features as described in standard parasitology sources (Garcia, 2009; Gunn and Pitt, 2012). The entire 0.2 g sample was viewed on a series of slides. Egg counts per gram of soil were calculated by counting the number of eggs observed across all slides for a given sample, and then multiplying this number by 5.

Disaggregated sediment that passed through the 20 μm sieve was collected and analyzed for the presence of protozoa that cause dysentery, specifically *Entamoeba histolytica*, *Giardia duodenalis*, and *Cryptosporidium parvum*. Eight subsamples from each latrine sample were tested with Enzyme-Linked Immunosorbent Assay (ELISA) test kits specifically designed by Techlab© to detect the cysts of these organisms, which range in size from 2 to 20 μm. They employ a monoclonal antibody-peroxidase conjugate specific for proteins uniquely secreted by these organisms, with reported 100% sensitivity and 99.8–100% specificity (Sharp et al., 2001). The ELISA reader used was a BioTek Synergy HT Multi-Mode Microplate Reader, with measurements taken at a wavelength of 450 nm. This ELISA analysis was then repeated at a later date to ensure reproducibility of our results.