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Abstract

To improve our knowledge of the parasite species affecting the inhabitants of Roman period Asia Minor, we analysed faecal material from Ephesus, Turkey. Mineralised material from the drain from a private house latrine (3rd c. CE), sediment samples from the sewer drain of a public communal latrine (6th c. CE), and sediment from the harbour canal (ca. 1st c. BCE to ca. 6th c. CE) were studied for the presence of intestinal parasites. Samples were viewed by light microscopy for helminth eggs, and commercial enzyme-linked immunosorbent assay (ELISA) kits were used to test for protozoal parasites that cause dysentery. Eggs of roundworm were found in the public latrine, whipworm in the house latrine, and both whipworm and roundworm in the harbour canal. Sequential sampling of the harbour core suggests that whipworm was by far the most common parasite throughout the Roman period, and there was no clear evidence for change in parasite species over the centuries. Whipworm and roundworm are both spread by the contamination of food and drink by human faeces. Despite the large number of travellers to Ephesus, as the capital of its province and a major port city in the Roman Empire, there was a surprising lack of diversity in parasite species found. This is especially apparent when we consider that ten species of intestinal parasite have been found across the Roman Empire. This is the first Roman site to be directly assessed for differences between infection in individuals using private latrines, public latrines, and mixed town effluent (in the harbour) at the same site.

Keywords: Palaeoparasitology; Ephesus; Roman empire; Helminths; Roundworm; Whipworm

1. Introduction

There is a fair range of archaeological and historical evidence for infectious disease in the Roman period. Particular focus has been placed on major pandemics that are known to have killed large portions of the population, including the Antonine Plague, the Plague of Cyprian, and the Plague of Justinian (Retief and Cilliers, 2000; Little, 2007; Wagner et al., 2014; Harper, 2015; McCormick, 2015). Through the study of skeletal remains there have also been accounts of other infections that can produce lesions on the skeleton including tuberculosis, treponemal disease, and leprosy (Roberts and Cox, 2003; Canci et al., 2005; Lewis, 2011; Minozzi et al., 2012; Rissech et al., 2013; Müller et al., 2014; Rubini et al., 2014). Most of the evidence we have for infection in the Roman period comes from studies focusing on bacteria. However, evidence for parasitic infection in the Roman period is steadily growing.

Parasites are one of the four main categories of pathogens, alongside bacteria, viruses, and fungi. The two main types of parasites are endoparasites, those that live inside the body, and ectoparasites, which live outside the body. Endoparasites, such as intestinal worms and single celled protozoa, are of particular interest to palaeoparasitologists studying disease in past populations because they offer insights into human migration, diet, sanitation, and interaction with animals (Reinhard, 1992; Le Bailly and Araújo, 2016; Araújo et al., 2008; Mitchell, 2013). Archaeological evidence for parasites in the Roman period has suggested that intestinal parasites were common contributors to disease in Roman populations in many areas of the Empire (Mitchell, 2017). This is despite the advent of centralized sanitation measures and widespread use of latrines and sewers during the Roman period, which are measures that are supported by modern epidemiological studies to decrease the incidence of parasitic infection (Feachem et al., 1983; Ziegelbauer et al., 2012; Speich et al., 2016).

The maximum expansion of the Roman Empire was reached in the 2nd century CE, when its borders extended up to northern Britain in the north, the Atlantic Ocean in the west, northern Africa in the south, and the Euphrates river in the east. Parasitic evidence from the Roman province of Asia (covering most of modern day western Turkey), is slim compared to other provinces, especially those in northern Europe. In fact, only one other archaeological site from Turkey has ever been studied for parasitic infection, the Roman period site of Sagalassos (Williams et al., 2017). This study aims to contribute to evidence of parasitic infection in Roman period Asia Minor through analysis of sediment samples from a public multi-seat latrine, a private house latrine, and a sediment core from the harbour canal at Ephesus. Using these different archives, we hoped to detect variations in parasite infection among the populations of the city.

2. Roman period Ephesus

The city of Ephesus (Fig. 1) has been well studied both from a historical and archaeological perspective due to its impressive ruins and religious history. It is the site of the famed Temple of Artemis and the library of Celsus. Ephesus has a long history of occupation beginning in the 7th millennium BCE. The Hellenistic city was founded by one of Alexander's successor, Lysimachos, in 296 BCE, and became part of the Roman Republic in 133 BCE (Ladstätter et al., 2016). Under the reign of Augustus, Ephesus was declared the capital of the province of Asia, in place of the former capital Pergamum, after which the city grew extensively through the Imperial period (Scherrer, 2001). It was situated along roads connecting the Aegean coast to the interior of Asia Minor as well as the Persian Royal Road and trade routes through the Meander valley (Ladstätter, 2018). It was also well connected to the rest of the Mediterranean region by its harbour. Historical records give further evidence for many high-status visitors who came to Ephesus for political reasons. As the seat of the proconsul in Asia and a centre for early Christian communities, it was a centre for commerce, politics, and scholarship, particularly in medicine.

Archaeological excavations began at the site in 1863 by J.T. Wood in his search for the Temple of Artemis. Since 1895 excavations have been continued by the Austrian Archaeological Institute, uncovering public and private spaces of the Late Antique/Byzantine city and harbour area (Ladstätter et al., 2016). Sediment samples collected during recent excavations were used to look for evidence of parasitic infection in the population of the city (Fig. 2).

3. Materials and methods

The first set of sediment samples were collected from the drain of a public latrine associated with the adjacent Varius bath complex, also called the Baths of Scholastikia

(Jansen, 2006). This bath complex was located along a major public street through the ancient city. The public latrine was available for use by anyone visiting the baths. This means it would have attracted not only locals but also those from other provinces within the Empire who were visiting Ephesus for trade, politics, scholarship, or passing through on their way to other cities. Sediment samples were taken at four depths from the surface (0–20 cm, 40–60 cm, 90–110 cm, and at 150 cm) in the drain of the public latrine, after modern sediment had been removed (Fig. 3). All samples date to the 6th century CE, the latest period of use of the latrine.

The second set of samples were collected from a private latrine (Room No. 34/34a) of one of the nearby Terrace houses, dated from the Augustan period to the 3rd century CE (Ladstätter, 2013; Ployer, 2016). Archaeological excavation of the Terrace houses at Ephesus has shown that they were quite opulent and would have been inhabited by higher class individuals who were able to afford the luxury of a private latrine. Mineralised material adherent to the sides of the sewer connected to the latrine from Terrace House 2 (unit 7) was scraped off and used for analysis (Fig. 4b). The seating component of the latrine was destroyed in the 3rd century CE (Fig. 4a).

The third set of samples were collected from a sediment core (Eph 244) from the harbour canal, which connected the Roman harbour basin of Ephesus with the open sea of the Aegean (Fig. 2). The canal was built to maintain access to the city for shipping, as the harbour tended to silt up with sediment from the Caystros (modern name: Küçük Menders) delta. We analysed sequential sections of the sediment core dating from the 1st century BCE to the 6th century CE. We anticipated that sewers and drains from the city would open into the waterfront, thus carrying the eggs of intestinal parasites into the harbour. Results of the sediment core analysis for microfauna, pollen, spores, plant fragments, and shells were published elsewhere, and mention was made of the parasite species present in the sediment (Stock et al., 2016). The dates for all these samples have been determined from an intensive series of radiocarbon dates that have been undertaken across the excavation at Ephesus.

Samples were analysed in the Palaeoparasitology Lab at the University of Cambridge, UK. A 0.2 g subsample was first disaggregated (made into a liquid suspension) to release parasite eggs from other material in the sample. For the public latrine sediment, 0.5% aqueous trisodium phosphate was used for disaggregation. After a period of 2 hours with periodic shaking of the test tube, the sediment was completely disaggregated. The resulting suspension was poured through a stack of microsieves with mesh sizes of 300 µm, 160 µm, and 20 µm on top of a catchment container. The typical range for the dimensions of human parasitic worm eggs is between 22 µm (width) and 140 µm (length) (Garcia, 2016). Therefore, any parasite eggs in the sample would have been trapped on top of the 20 µm sieve. This material was collected and centrifuged at 4,000 rpm for 5 minutes. The supernatant was removed and the remaining material mixed with glycerol and viewed at 400× magnification on a light microscope equipped with a digital camera (Anastasiou and Mitchell, 2013).

For the mineralised material from the private latrine of Terrace House 2, 0.5% trisodium phosphate was not sufficient to break up the concretions. Dilute hydrochloric acid (HCl) was used to breakdown calcium carbonates to release parasite eggs from the concretion. HCl was added dropwise to the sample until a reaction occurred, evident by gentle bubbling due to production of carbon dioxide. Each time the reaction stopped, HCl was added dropwise to continue the reaction until the sample was completely disaggregated. The sample was centrifuged and washed with trisodium phosphate five times to dilute and wash out any remaining HCl. It was then mixed with glycerol and viewed under the microscope. Disaggregation using HCl has been evaluated in comparison to other reagents, and it was observed that HCl can decrease species diversity and should only be used when material cannot be processed using typical non-destructive solutions (Dufour and Le Bailly, 2013).

Each sample was also tested for single-celled protozoal parasites that cause dysentery, including *Entamoeba histolytica*, *Giardia duodenalis*, and *Cryptosporidium parvum*, using enzyme-linked immunosorbent assay (ELISA). ELISA is an immunological method that tests for the presence or absence of specific organisms using a plate with wells containing immobilized antibody specific to the pathogen in question. If the pathogen, or the specific antigen being targeted, is present it binds to the antibody in the wells. Another antibody linked to an enzyme is then added which binds to this complex and causes a visible colour change, which confirms the presence of the pathogen. Commercial ELISA kits from TechLab©, designed and tested on clinical samples employing a monoclonal antibody-peroxidase conjugate specific for proteins uniquely secreted by these organisms, were used for analysis. The sensitivity and specificity for each of the kits is both 100% for *Giardia* *duodenalis*, 96.9% sensitivity and 100% specificity for *Entamoeba histolytica*, and 97.7% sensitivity and 100% specificity for *Cryptosporidium parvum* (Sharp et al. 2001). For each sample, the disaggregated and sieved sediment from underneath the 20 µm sieve was collected and used for the tests, as the protozoal cysts are typically less than 20 µm in size (Garcia, 2016). The ELISA tests were carried out following the manufacturer's procedure. A negative and positive control were present on each ELISA plate, and an ELISA plate reader was used to quantify colour changes in each well.

4. Results

Results from the microscopy revealed parasite eggs in all samples studied. Samples from the public latrine contained eggs of roundworm (*Ascaris* sp.). These eggs were found in samples from all four depths, although a higher concentration of eggs were found in the middle two layers, from 40–60 cm and 90–110 cm. A total of twenty fertilized roundworm eggs were found in the samples from the public latrine (Fig. 5). Two subsamples were studied from the middle two layers to ensure that no species were missed on analysis; this gave a concentration of 17 eggs per gram in the latrine. This suggests that the egg concentration was not high. The typical size range of modern *Ascaris lumbricoides*, the species that infects humans, is 40–75 µm long and 35–50 µm wide (Garcia, 2016). The eggs found in the public latrine from Ephesus had mean dimensions of 60.9 µm long and 46.5 µm wide (Table 1). All of the roundworm eggs found were completely or partially decorticated, having lost their outer mammillated coat. This is quite common for eggs found from archaeological contexts, as the mammillated coat on the outside of the chitinous wall of the eggs is more susceptible to degradation by taphonomic processes (Rácz et al., 2015). When the eggs are decorticated, their dimensions will be a few µm smaller than when the mammillated coat remains.

Eggs of human whipworm (*Trichuris trichiura*) were found in the mineralised material from the private latrine at Terrace House 2 (Fig. 6). A total of 11 eggs were found in 0.2 g of material, a concentration of 55 eggs per gram. The mean length of eggs found was 50.1 µm and the mean width was 26.4 µm (Table 1). The typical size range of whipworm eggs is 34–57 µm long without polar plugs, or 49.5–65 µm long with polar plugs, and 20–30 µm wide (Beer, 1976; Garcia, 2016).

Samples from the harbour canal sediment core were found to contain the eggs of both roundworm and whipworm (Fig. 7). We analysed sediment dated to ca. the 1st century BCE to ca. the 6th century CE, spanning the entire time period of the other samples studied in the city. Nine samples were taken at regular intervals from along the core. While both whipworm and roundworm eggs were found in the sediments, they were in a ratio of 19:1 whipworm to roundworm. Whipworm was found in every sample throughout the Roman period.

Parasite	Sample Location	Date	No. of eggs	Length (µm)			Width (µm)		
				Range	*Mean*	*σ*	*Range*	*Mean*	*σ*
Roundworm	Public Latrine	6th c. CE	20	54.6–66.4	60.9	3.4	40.3–51.7	46.5	2.8
Whipworm	Terrace House Latrine	3rd c. CE	11	44.8–55.7	50.1	3.6	24.2–28.0	26.4	1.2

Table 1. Location of parasite eggs found in latrines at Ephesus, parasite egg counts, size range, mean dimensions, and standard deviations (σ).

ELISA tests were negative for all three protozoa, *Giardia duodenalis*, *Entamoeba histolytica*, and *Cryptosporidium parvum*. The positive and negative controls had the appropriate colour change and absorbance values to confirm that the test was undertaken properly and there were no design errors in the plates.

5. Discussion

We have investigated parasitism in the Roman period population of Ephesus by analysing complementary samples from a communal public latrine, a private house latrine, and the runoff from city drains and sewers into the harbour. We found good evidence that whipworm and roundworm infected some members of the population.

Roundworm (*Ascaris* sp.) is one of the most common intestinal worms both in the past and today. It is transmitted via the faecal-oral route when eggs of the worm polluting soils and water are ingested. When a human is infected with roundworm, the worm releases approximately 240,000 eggs per day in that individual's faeces (Weller and Nutman, 2014). These eggs then mature in the external environment over a period of 2 weeks at which point they become infective, and upon ingestion by the same or another individual they can develop into adult worms in the small intestine. The adult roundworm is 15-30 cm long and can live for 2 years (Jourdan et al., 2017), absorbing nutrients from the gut before they can be absorbed for use by the individual. The symptoms experienced by an infected individual vary based on age, comorbidities, health status, and worm burden (the number of worms present at one time). Roundworm infection may be asymptomatic, but infection can be particularly detrimental to children and those with a high worm burden resulting in anaemia, abdominal emergencies such as intestinal obstruction, pancreatitis, cholecystitis, and liver abscess (Jourdan et al., 2017). Common symptoms include abdominal discomfort, altered bowel habits, decreased energy, and weight loss.

Whipworm (*Trichuris trichiura*) is a soil-transmitted helminth, like roundworm. An infected individual sheds eggs in their faeces. These eggs mature in the soil and infect new individuals when they are ingested. The adult whipworm grows to around 3 cm long in the large intestine and colon (Garcia, 2016). The worm releases on average 5,000 eggs per day and lives for a period of up to 5 years (Weller and Nutman, 2014). Whipworm burrows into the mucosa of the intestine and can cause inflammation and bleeding; as a result, anaemia can be more severe with whipworm infection compared to roundworm infection (Jourdan et al., 2017). Similar to roundworm, symptoms include abdominal pain, change in bowel habits, weight loss, and with a higher worm burden, rectal prolapse.

Sanitation measures such as latrine use and hand washing have been shown to significantly decrease the transmission of whipworm and roundworm (Zeigelbauer et al., 2012). However, despite the relatively low concentration of eggs noted, it appears that latrines did not fully protect the population at Ephesus. This could be due to a number of reasons. It is unlikely that everyone in Ephesus would have used latrines, and in houses that did not have their own private latrines faecal material would have been collected and dumped into open sewers, streets, or directly into wagons to be taken away (Taylor, 2015). Similarly, those without access to latrines when they needed them may have defecated elsewhere in the city, such as in streets, doorways, and behind statues (Scobie, 1986). In this environment, one could come into contact with human excrement as they ventured through the city on any number of errands. Furthermore, although there is evidence for running water in the large public latrines which was available for hand washing after use of the facilities, we do not know to what extent hand hygiene was emphasized. For those individuals who relieved themselves in the streets, the public fountains may have also served the purpose of a place to rinse hands. Many people living in large cities would have collected drinking water from public fountains or basins which could have been contaminated by these dirty hands, and containers used to collect water (Scheidel, 2009). Finally, human faeces were known to be an effective fertilizer for crops in the Roman Empire. There are numerous written records and archaeological evidence attesting to the use of human faeces as fertilizer after it was collected from Roman cities (Farmer, 1918; White, 1970; Baeten et al., 2012; Koloski-Ostrow, 2015). This process offers an ideal route for transmission of intestinal worms when crops contaminated with infective parasite eggs from fresh human faeces are then eaten by a high proportion of the population.

Ephesus was a major trading center in Roman Asia Minor. As such it is interesting that we did not find any evidence for other intestinal parasite species that have previously been identified in the Roman Empire. Besides roundworm and whipworm, the lancet liver fluke (*Dicrocoelium dendriticum*), *Taenia* tapeworms, fish tapeworm (*Diphyllobothrium* spp.), *Fasciola* liver fluke, *Capillaria* spp., pinworm (*Enterobius vermicularis*), and protozoa causing dysentery (*Giardia duodenalis* and *Entamoeba histolytica*) have all been found in faecal samples studied from Roman period sites (Rouffignac, 1985; Horne, 2002; Harter, 2003; Le Bailly and Bouchet, 2010; Le Bailly and Bouchet, 2013; Mowlavi et al., 2014). There was also the potential to find the eggs of schistosomes from those people who travelled to Ephesus from Africa or the Middle East (Anastasiou and Mitchell, 2015). Currently, the majority of data that exists for parasites in the Roman Empire come from sites that have been studied in northern Europe, especially France and Britain. In these areas, a more diverse range of species has been found in archaeological material compared to the Mediterranean region.

The lack of parasite species diversity at Ephesus makes us consider options for why this might be the case. One possibility is that animal meat may have been well cooked before it was eaten, killing parasite larvae in the meat and so preventing infection. Historical evidence suggests that eating raw meat was unusual in hotter regions of the Roman Empire and that cooking processes adopted from the Greeks relied on boiling meat which could then be followed by roasting (Chandezon, 2015). This extensive cooking process would allow the meat to reach temperatures that would kill parasites in pork or beef, effectively removing the risk for *Taenia* tapeworms. This is in contrast to northern Europe in the past, where there was more of a tradition of eating raw, pickled, dried or salted animal products (Mitchell, 2017). Another possibility is that the diet of those at Ephesus was highly dependent upon farmed foods rather than wild foods hunted and caught from the countryside. This may explain the absence of zoonotic parasites at Ephesus that are spread by the consumption of these wild animals.

A further issue to consider is that we failed to detect parasite species that genuinely infected the population because they did not survive well in these archaeological contexts. The hot dry climate of the Mediterranean region may not be as conducive to the survival of parasite eggs compared with cooler, moister archaeological soils in northern Europe, for example. The effect of taphonomy is most likely to be relevant for species with thin and fragile eggs, such as pinworm or hookworm (Bouchet et al., 2003). The absence of protozoa causing dysentery is unlikely to reflect genuine absence of dysentery in the population throughout the Roman period, as ELISA tests for *Giardia duodenalis* were positive from Roman period Sagalassos, indicating that this organism was present in the region at the time (Williams et al., 2017). It is possible that protozoa were present in at least some of the population at Ephesus, but that the samples we analysed did not contain a high enough concentration of antigen to trigger a positive result for *Giardia*. There is also evidence for *Entamoeba histolytica* in the Roman period from sites in Belgium, France, and Italy (Le Bailly and Bouchet, 2015). We should consider that the HCl needed to disaggregate the mineralised samples from the terrace house latrine may potentially have interfered with the ability of the ELISA kits to detect protozoa if they were present.

A final factor we should discuss is the potential use of anti-helminthic medicines by local medical practitioners. Galen of Pergamum (130-210 CE) was a medical practitioner from Asia Minor, who described several types of intestinal helminth in his medical works and recommended a range of medical treatments to expel them (Jirsa and Winiwarter, 2010). A number of those medicines have been shown to have anti-helminthic effects in laboratory studies and when given to animals infected with helminths (Githiori et al., 2006; Idris et al., 1982; Khan et al., 2015; Mahmoud et al., 2002; Molefe et al., 2012). However, the degree to which these medicines may have been used in Roman times, and how effective they may have been in humans, is very hard to evaluate using archaeological evidence as we have here.

Palaeoparasitological analysis of multiple latrine sediments from Roman Ephesus allows us to explore the distribution of parasites from a single site. Interestingly, the parasites found in the public and private latrine from the site showed no overlap. Roundworm was found alone in the public latrine, and whipworm was found alone in the private house latrine. We expected to find a low number of species in the house latrine due to the small number of people using it, while we expected a larger number of species in the communal public latrine, as large numbers of people and likely more visitors would have used this every day. However, only one species, roundworm, was found in the sewer drain of the public latrine. Since we studied multiple samples from the drain, this would suggest that the population was genuinely not infected by a broad range of parasites. This is confirmed by the fact that in the harbour sediment, where all the city drains and effluent eventually ended up, only whipworm and roundworm eggs were found.

Currently 10 species of intestinal parasite have been identified in Roman period sites across the empire. Of these species, whipworm and roundworm were the most common. Whipworm and roundworm have been found together at Roman period sites in Austria, Belgium, Britain, France, Germany, Greece, Israel, and the Netherlands (Anastasiou et al., 2017; Aspöck et al., 1999; Bouchet et al., 2001; Boyer, 1999; Gourevitch et al., 2011; Jansen and Over, 1962; Jones, 1985; Jones, 1987; Jones and Hutchison, 1991; Knights et al., 1983; Kuijper and Turner, 1992; Pike, 1968; Rouffignac, 1985; Rousset et al., 1996; Wilson and Rackham, 1976; Zias et al., 2006). However, there are also four sites where roundworm has been found without whipworm (Dittmar et al., 2002; Harter, 2003; Aspöck et al., 2011; Williams et al., 2017), and six sites where whipworm has been found without roundworm (Witenberg, 1961; de Moulins, 1990; Carrott et al., 1995; van Geel et al., 2003; Heirbaut et al., 2011; Dufour et al., 2016). The sample type does not appear to affect the preservation of these two parasites, as both species occur in latrine sediment, mummified remains, pelvic soil, and occupation layer sediment. Based on other work undertaken on Roman period samples, it appears that it is most common to find both species preserved together, but there are a substantial number of cases where roundworm or whipworm occur without the other. This may indicate that in certain environments, one parasite was more effective than the other at infecting the population.

Study of the harbour sediment core provides the opportunity to look for change over time in the type of parasites present. The eggs of whipworm were found in every core dating from the Roman period, with occasional roundworm. We did not detect any change in the species of parasite throughout the Roman period. However, it is interesting that far more whipworm eggs were found in the harbour sediment than was the case for roundworm, with the ratio being 19 to 1. Modern roundworms produce about 240,000 eggs per day in that individual's faeces, and whipworms produce far fewer eggs, around 5,000 eggs per day (Weller and Nutman, 2014). As there is no reason to believe that roundworm eggs are less likely to survive in archaeological contexts than whipworm eggs, this would suggest that over time there were far more whipworms than roundworms producing eggs in the population of Ephesus.

6. Conclusions

We studied different samples from across Ephesus using microscopy and ELISA to determine both the species of intestinal parasite that infected the population, as well as any differences in infection between people using different latrines. Analysis of the public latrine revealed human roundworm, while human whipworm was found in the private house latrine. The dominance of whipworm eggs over roundworm in sequential sampling of a sediment core from the Roman harbour suggests that whipworm was probably the most common intestinal parasite in the inhabitants throughout the Roman period, and there was no clear evidence for change in parasitism over time. The fact that both these species of parasite are spread by the faecal contamination of food and drink highlights that sanitary issues were the dominant factor resulting in the spread of parasites in this population. The results were compared to what is known about parasitic infection in the rest of the Roman Empire, as well as the one other archaeological site that has been studied in Turkey (Sagalassos). The results from those sites so far studied in Roman period Asia Minor do appear to contrast with those from northern Europe during the same period where many more species were present. This may be due to differences in climate, diet, cooking, medical practices, and the preservation of parasite eggs in different archaeological environments.

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(a)

(b)

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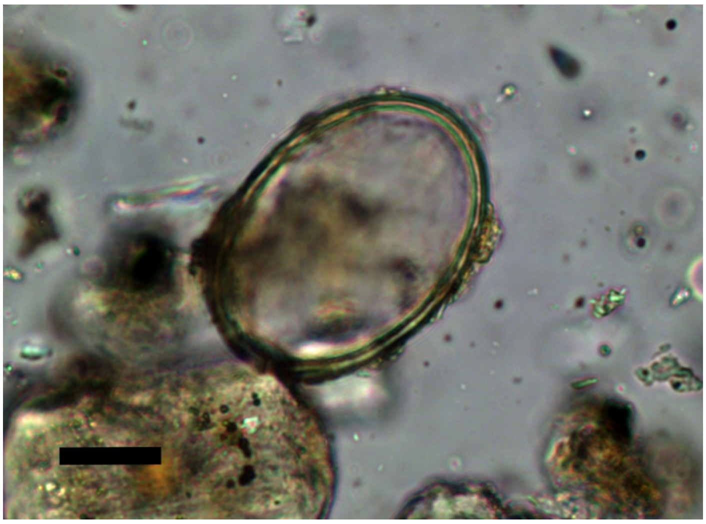

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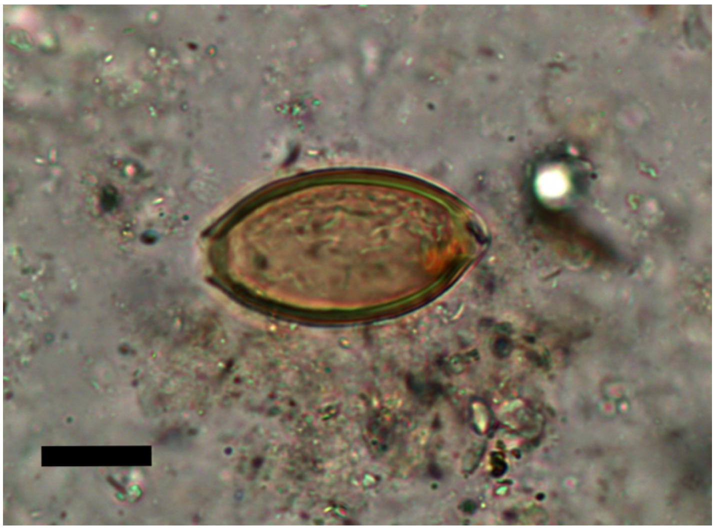

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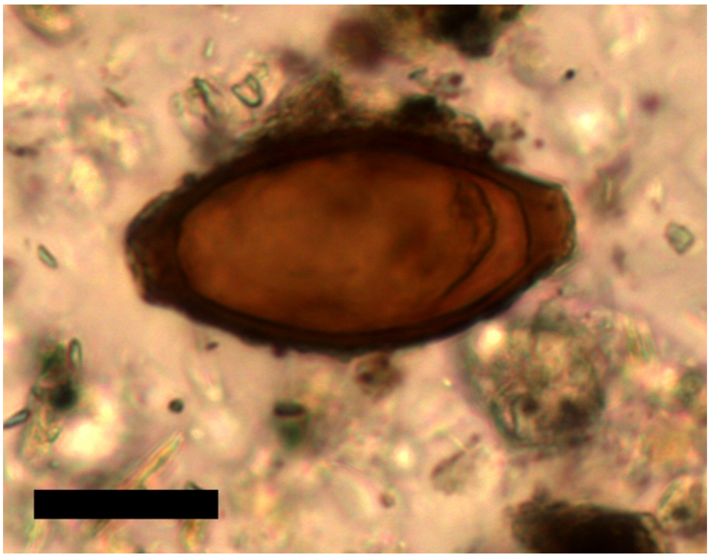

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