Gastrointestinal infection in Italy during the Roman Imperial and Longobard periods: A paleoparasitological analysis of sediment from skeletal remains and sewer drains

Marissa L. Ledger [a],[*], Ileana Micarelli [b], Devin Ward [c], Tracy L. Prowse [d], Maureen Carroll [e], Kristina Killgrove [f], Candace Rice [g], Tyler Franconi [g], Mary Anne Tafuri [b], Giorgio Manzi [b], Piers D. Mitchell [a]

[a] Department of Archaeology, University of Cambridge, The Henry Wellcome Building, Fitzwilliam Street, Cambridge, CB2 1QH, UK
[b] Department of Environmental Biology, Sapienza University of Rome, Piazzale Aldo Moro 5, 00185, Roma, Italy
[c] Department of Anthropology, University of Toronto, ON, Canada
[d] Department of Anthropology, McMaster University, Hamilton, ON, Canada
[e] Department of Archaeology, University of York, UK
[f] Department of Anthropology, University of North Carolina at Chapel Hill, USA
[g] Joukowsky Institute for Archaeology and the Ancient World, Brown University, USA

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ABSTRACT

Objective: This study aims to investigate parasitic infection in Italy during the Roman period (27 BCE–476 CE) and subsequent Longobard (Lombard) period (6th–8th CE).
Materials: Sediment samples from drains and burials from Roman Imperial-period sites in Italy (Lucus Feroniae, Oplontis, Vacone, and Vagnari), Late Antique and Longobard-period burials at Selvicciola (ca. 4th–8th CE), and Longobard-period burials at Vacone and Povegliano Veronese.
Methods: Microscopy was used to identify helminth eggs and enzyme-linked immunosorbent assay (ELISA) to detect protozoan antigens.
Results: Roundworm and whipworm were found in pelvic sediment from Roman-period burials, while roundworm and the protozoan *Giardia duodenalis* were identified in Roman-period drains. In pelvic sediment from the Late Antique through Longobard periods, roundworm and *Taenia* tapeworm eggs were identified.
Conclusions: Fecal-oral parasites were found throughout Imperial Roman Italy, suggesting that gastrointestinal infections caused a significant disease burden. In the Longobard period we see continuity in transmission of fecal-oral parasites, and the appearance of zoonotic parasites acquired from eating undercooked meat.
Significance: A wealth of information exists about certain diseases in the Roman period, but relatively little is known about intestinal parasites in Italy during the Roman and Longobard periods. This is the first evidence for *Giardia* in Roman period Italy, and for any parasites in the Longobard period in Italy.
Limitations: Low egg concentrations and lack of controls for some samples makes it difficult to differentiate true infections from environmental contamination in some cases.
Suggestions for future research: Continual study of samples from Roman and Longobard period Italy.

1. Introduction

Today, one of the leading causes of mortality in children under five is diarrhea, and in 2016, it was the eighth leading cause of mortality among those of all ages (Global Burden of Disease 2016 Diarrhoeal Disease Collaborators, 2018). A majority of diarrheal illnesses are caused by enteric pathogens including viruses (e.g. norovirus and rotavirus), bacteria (e.g. *E. coli* and *Salmonella*), and parasites (Fletcher et al., 2013). Gastrointestinal diseases are likely to have been major causes of morbidity and mortality in the human past, as well. Despite this, we know little about gastrointestinal disease in Roman Period Italy. Detecting viral and bacterial gastroenteritis in ancient populations is

challenging due to the acute nature of most infections, a lack of skeletal changes, and difficulties extracting and identifying pathogenic viral and bacterial DNA in fecal samples. Compared to viruses and bacteria, there are large numbers of studies identifying enteric parasites in archaeological samples (Mitchell, 2015), which provide important insights into the burden of gastrointestinal disease in past populations. The presence of specific parasite taxa permits identification of societal and environmental conditions that likely contributed to their spread and the spread of related pathogens. For example, the presence of parasites spread by the fecal-oral route prompts consideration of sanitation and hygiene levels, whereas the presence of foodborne parasites may lead to investigation of human-animal interactions and dietary practices that could lead to those infections.

The Roman period is a valuable time to examine how territorial expansion and increased migration resulted in variations in disease burden. As the Empire expanded and incorporated new regions within its borders, the existing cultural practices and identities of these groups were not forcibly abandoned, but maintained and incorporated with new Roman influences resulting in regional variations in ways of life across the Empire (Woolf, 1997; Keay and Terrenato, 2001; Garnsey and Saller, 2015). What is often referred to as a typical 'Roman' way of life is based upon what is known about those living in the Mediterranean region, and Italy in particular, during the Imperial period. However, even in Italy the 'Roman' way of life changed through time as new regions were conquered and individuals from distant regions of the Empire moved into Italy (Woolf, 1997; Cooley, 2016). This variation makes it important to investigate regional differences in disease experience within the Empire, to allow us to move away from a single summarized view of parasitic infection in the Roman period (Dufour, 2015; Ledger et al., 2020).

During the period of the Republic, Rome expanded its control from central Italy to the entirety of the Italian peninsula after a series of wars in the 5th–3rd c. BCE (Scopacasa, 2016). At the end of the Republic, with Augustus established as the first emperor in 27 BCE, Italy became the epicentre of the newly formed Roman Empire. As the Empire continued to expand during the Imperial period, Italy remained a relatively stable and prosperous area with continual benefits from trade within the Empire and beyond (Cooley, 2016). It is at this time that movements of people, widescale trade of goods and ideas, increases in population size, and urban expansion were occurring, especially in large cities such as Rome, all of which are known to have profound impacts on infectious disease and parasite transmission.

With increased political instability in the 3rd c. CE, it became difficult to simultaneously maintain control of all of the frontier regions of the Empire; thus at the end of the century, the Empire was split into the Western and Eastern Empires governed by separate emperors. This time period of instability marks the start of what is often referred to as the Late Antique period. Italy was part of the Western Roman Empire. The Western Roman Empire dissolved in 476 CE in the midst of a prolonged period of instability. This period has been characterized by migration of large groups of people, including Goths, Vandals, Anglo-Saxons, and Longobards, into areas that were previously part of the Empire (Goffart, 2006). The Longobards were Germanic peoples who first moved into the Roman province of Pannonia (corresponding to modern-day western Hungary, the eastern Austrian border, northern Croatia, and part of Slovenia) and then carried on into Italy (Alt et al., 2014). The Longobards invaded Italy in 568 CE and came to control much of northern and central Italy for the next two centuries (Amorim et al., 2018).

With migrations of large numbers of people such as the Longobards into Italy in the 6th century CE, we hypothesize that parasites were carried with them; especially with the first generation of migrants who could have had parasites in their intestines as they moved. Since many helminths can survive in human hosts for multiple years (Garcia, 2016), these parasites could have easily moved with migrating individuals and then been maintained in their new environment. Previous paleoparasitological studies in the Roman period and preceding time periods

have shown that there were more zoonotic parasite species being transmitted in areas of northern Europe, from where the Longobards would have migrated, compared to what has been found in Italy during these time periods so far (Ledger et al., 2020). For example, beef/pork tapeworm (*Taenia* sp.) has been found at the Roman site of Carnuntum in Austria (Aspöck et al., 2011; Petznek, 2018), and in multiple Roman sites in Germany along with *Capillaria* (Dittmar et al., 2002). Changes in cultural practices (e.g. animal husbandry, waste removal, diet) may also contribute to different parasites seen in the Longobard-period individuals compared to Roman individuals, making samples from Longobard-period sites very useful for studying potential changes in disease transmission with migration and changing cultural practices.

The aim of this study was to characterize parasitic infection in both the Roman Imperial and Longobard periods in Italy in order to identify continuity or change in parasite transmission over this transitional period. Identification of parasite taxa present is then used to explore factors that allowed for spread of gastrointestinal pathogens in Italy during the Roman period, and taxonomic diversity is compared to what has been found in other areas of the Roman Empire.

2. Materials and methods

2.1. Materials

Diagnosis of intestinal parasitic infection relies on analysis of fecal samples for helminth eggs and protozoal cysts. Worms and protozoa reproduce within the intestines and release eggs or cysts into the feces that can then be transmitted to another host. For this reason, we collected sediment samples from areas where fecal material would be found. Two main types of samples were collected; 1) sediment from the pelvis of skeletons where the intestines would have decomposed; and 2) sediment from the fill of sewer drains or latrines. The use of these two types of samples is valuable because the pelvic soil can provide information about infection at the individual-level, whereas the sewer and latrine samples provide evidence at the community-level, detecting infection in anyone in the community who may have been using this infrastructure. These samples came from six different sites dating to the Roman through the Longobard period in Italy (Fig. 1). Samples dating to the Roman Imperial period came from Lucus Feroniae (1st–3rd c. CE), Oplontis (79 CE), Vacone (1st–2nd c. CE), and Vagnari (1st–4th c. CE); while samples dating to the Longobard period came from Povegliano Veronese (6th–8th c. CE) and Vacone (7th–8th c. CE). Additionally, samples from Selvicciola (4th–8th c. CE) covered the Late Antique period through the Longobard period (see Appendix for details of samples collected from each site).

2.1.1. Roman-period samples

Lucus Feroniae is a town located 30 km northeast of Rome that was re-founded as a veteran colony in the later first century BCE (Patterson et al., 2020). The cemetery of the *via Capenate* associated with the site was excavated in the 1980s and the human remains are housed in the Museo di Antropologia at Sapienza University of Rome (Gazzetti, 1986). It has been proposed that the individuals buried at the cemetery had a generally low socioeconomic status and may have been slaves, freedmen, and veterans (Manzi et al., 1989; Tafuri et al., 2018). The location of Lucus Feroniae would have meant easy access to Rome along major roads. Anthropological investigations of the skeletons have been undertaken and revealed the presence of lesions that are often attributed to metabolic stress, such as Harris lines and linear enamel hypoplasia (Argenti and Manzi, 1988; Manzi et al., 1989, 1997, 1999; Salvadei et al., 2001; Tafuri et al., 2018). As this was a museum collection, sediment adhering to the sacrum and from the sacral foramina of 14 skeletons was collected for analysis. Sediment from inside the cranium and adhering to the foot bones was collected as control samples. In cases where these controls could not be collected, a subsample of the general burial sediment sample taken at the time of excavation was used.
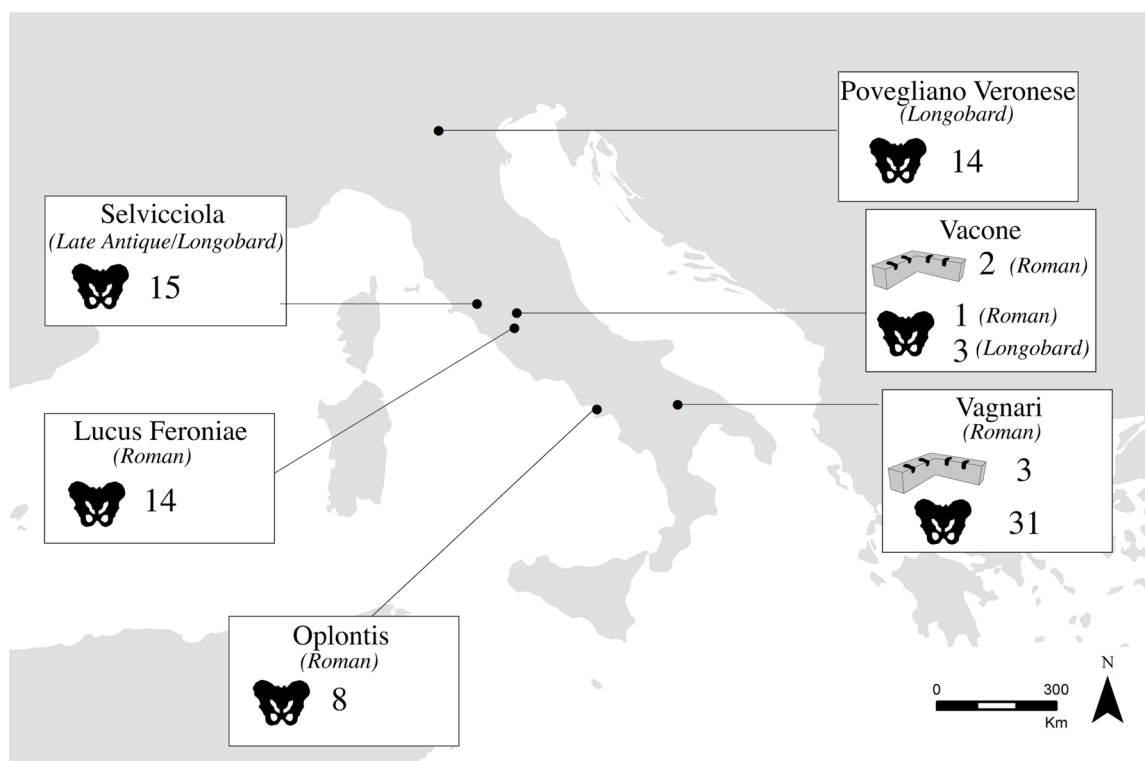
Povegliano Veronese
(Longobard)
14

Selvicciola
(Late Antique/Longobard)
15

Vacone
2 *(Roman)*
1 *(Roman)*
3 *(Longobard)*

Lucus Feroniae
(Roman)
14

Vagnari
(Roman)
3
31

Oplontis
(Roman)
8

0   300
Km

N

Fig. 1. Map of Italy indicating locations of sites where samples were collected. Inside each box the name of the site, time period of samples, and types of samples analyzed are given. The pelvis icon indicates that pelvic sediment samples were analyzed, and it is followed by the number of individuals sampled; the latrine icon indicates that latrine/drain samples were analyzed, and the number indicates the number of different drains or latrines that were sampled. From Vagnari and Vacone the number of different drains sampled are listed but multiple samples came from the length of these drains.

However, the location of sampling within the burial was not recorded for these general burial samples.

Oplontis, located in the modern city of Torre Annunziata, is an Imperial Roman site in the Bay of Naples, destroyed by the eruption of Mount Vesuvius in 79 CE. There are two main complexes at the site, referred to as Villa A and Villa B. Villa A is an elite villa and Villa B, despite its name, is not a traditional Roman villa, but consists of a central courtyard surrounded by two-storey buildings (Thomas, 2016; Van der Graaff et al., 2016). Some of these rooms contained large numbers of shipping jars (amphorae), which were likely used to transport wine and fish sauce, attesting to the trade connections of the villa (Muslin, 2016). In one of these rooms, the skeletal remains of more than 64 individuals were discovered, all of whom died during the eruption of Mount Vesuvius (Killgrove, 2018). Approximately half of the skeletons were fully excavated in 1984. Sediment from the pelvis of eight undisturbed individuals was collected during final excavation in 2017, and control samples were collected from each cranium. These sampled individuals were mostly adult females but also included a child and an adolescent.

The Roman villa at Vacone is located 55 km north of Rome in the small town of Vacone. The villa was built in the Late Republican period (2nd c. BCE), and was then extensively renovated in the early Imperial period (late 1st c. BCE to early 1st c. CE) (Franconi et al., 2019). The villa consists of numerous rooms for living and production. Early Imperial production facilities for olive oil have been identified, and these were later replaced by a mixed production of wine and oil. The villa was abandoned at the start of the 3rd century CE after it was damaged, possibly by an earthquake. Sediment samples were collected from two areas at the villa: a possible latrine located in Room 33 (three samples), and a *cappuccina* drain, which is a drain covered with roofing tiles in an inverted 'V' (control samples from the uppermost modern fill and four samples from the Roman-period fill taken at different points along the drain). Following its collapse, the villa was periodically used for burials. There are two distinct periods of burials: the late Roman period, shortly

after the collapse of the villa in the 3rd century CE, and the early medieval period, especially the 7th century CE. The late Roman burials discovered thus far are all non-adults, concentrated largely in one room of the villa (Room 50). Only one of the burials was sufficiently undisturbed to sample for paleoparasitological analysis (VAC 1 Grave 10).

Vagnari was an Imperial Roman estate in southern Italy, located near the modern-day city of Gravina in Puglia. The site consists of a *vicus* (village or town) and associated cemetery. The *vicus* at Vagnari was constructed in the area of a minor Iron Age site and became the centre of an Imperial Estate around the 1st century CE. Industries including tile production, iron working, lead processing, and wine production were established (Small, 2011; Prowse and Carroll, 2017; Carroll, 2019). There were a series of drains running underneath the buildings at the *vicus,* which are hypothesized to have carried waste or water run-off. Three drains were sampled for analysis. Two samples were collected from one of the drains and one sample from each of the other two drains. The cemetery at Vagnari was in use between the 1st–4th centuries CE. Around 160 burials have been excavated so far. All age groups are represented, and both males and females are present in roughly equal proportions. Stable isotope and mitochondrial DNA studies suggest that the majority of people buried at Vagnari were local or immigrated from nearby southern Italy (Prowse et al., 2010; Emery et al., 2018a, b). Sediment samples were collected from the pelvis of 31 skeletons, and control samples were collected from the cranium and the feet.

2.1.2. Late Antique and Longobard-period samples

Selvicciola (4th–8th c. CE) is a Late Antique and Longobard cemetery located in present-day northern Lazio. The skeletal remains are housed in the Museo di Antropologia at Sapienza University of Rome (Gazzetti, 1985, 1986; Micarelli, 2020). Some of the excavated individuals were originally identified as Longobard individuals based on funerary practices and grave goods. While the presence of grave goods indicates an influence of Longobard culture in the community, these individuals may

or may not have identified as ethnically Longobard and even those from local Italian populations may have been buried with these items. Others, based on stratigraphy and location in the cemetery, appear to be from the Late Antique period prior to Longobard migrations. Anthropological investigations have been undertaken and indicated that health may have declined in the Longobard period compared to the preceding Roman period (Salvadei et al., 1995; Sperduti et al., 1995; Salvadei et al., 2001; Micarelli et al., 2019). Sediment adherent to the sacrum and from the sacral foramina of 15 skeletons was collected. The individuals sampled are non-adults and adults, most are male but there is one female and others that could not be assigned to either sex. Two of those studied are from the Late Antique period and two are from the Longobard period. The remaining individuals could not be confidently dated, they could have been buried any time from the Late Antique period through the Longobard period (late 4th century to early 8th century). Control samples came from general burial samples that had been taken during excavation.

Povegliano Veronese is a Longobard cemetery located near present-day Verona. The skeletal remains are housed in the Museo di Antropologia at Sapienza University of Rome (Bruno and Giostra, 2012). The cemetery is thought to contain burials from a Longobard community founded shortly after their migration into Italy (Giostra, 2014). Strontium isotopes indicate that over half of the individuals buried in the cemetery were likely from the area around Povegliano Veronese, while around 30 % were non-local and had isotope signatures consistent with those from Hungary (Francisci et al., 2020). Anthropological investigation of the skeletal remains has been undertaken (Micarelli et al., 2018; Micarelli, 2020). Sediment adhering to the sacrum and from the sacral foramina of 14 skeletons was collected for analysis. Control samples came from sediment adhering to the cranium, the foot bones, or from general burial samples.

Longobard-period samples were collected from the villa at Vacone along with the Roman period samples mentioned above (Franconi et al.,

2019). After initial abandonment of the Roman villa at Vacone there was sporadic activity at the site over the next five centuries. While there is not yet evidence that the site was regularly inhabited in this later phase, a series of graves radiocarbon dated to the 7th–8th centuries CE were dug into the collapse of the villa. Though these burials date to the Longobard period, there is no evidence that the individuals were ethnically Longobard and were most probably from local populations who remained in the area after the Longobard invasions. Sediment from the pelvis of three individuals buried at the villa was collected for analysis.

2.2. Methods

Two main methods were used to detect parasites in the samples collected: 1) microscopy to detect preserved helminth eggs, and 2) ELISA to detect preserved protozoan antigens from three taxa: *Cryptosporidium* spp., *Entamoeba histolytica,* and *Giardia duodenalis.* All sediment samples were first rehydrated and disaggregated before being analysed by either method; the procedure for this and each subsequent method are described below and depicted in Fig. 2.

2.2.1. Disaggregation and microsieving

Subsamples from each sample were first taken; a 0.2 g subsample was used for microscopy and a 1 g subsample was used for ELISA. Subsamples were disaggregated (made into a liquid suspension) in 0.5 % trisodium phosphate to release preserved parasite eggs and cysts from the sediment matrix. Samples were vortexed periodically until all material was disaggregated, with sand particles and other insoluble materials floating freely. Once samples were adequately disaggregated they were sieved using a stack of microsieves with mesh measuring 300 μm, 160 μm, and then 20 μm. Distilled water was used to wash all material through each sieve. Sieves were cleaned between each use to prevent contamination between samples, and all other materials were single-use.
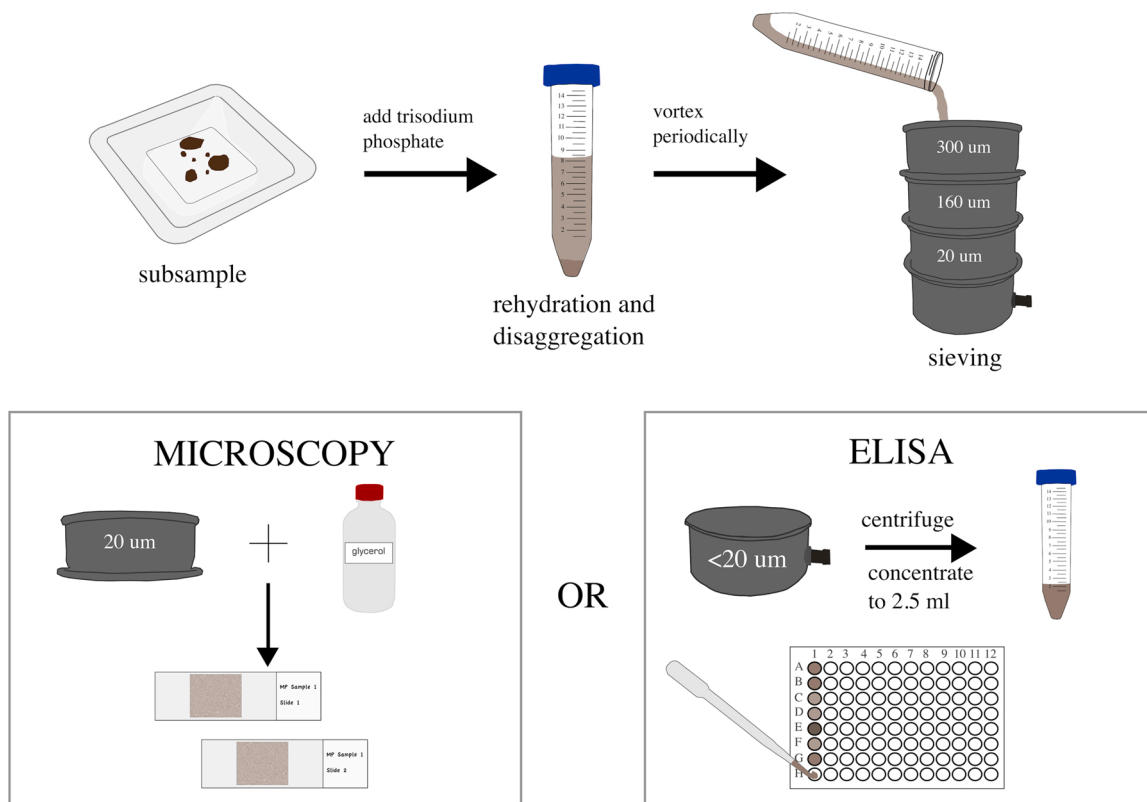

Fig. 2. Overview of methods used to analyze sediment samples for parasites. The upper images show disaggregation and microsieving, after which samples were analyzed using microscopy or ELISA, shown in the bottom half of the image.

2.2.2. Microscopy

For microscopy, the material trapped on the top of the 20 μm sieve was collected, as this is the proper size range to contain the eggs of helminth species endemic in this region of the world (Bouchet et al., 2001a; Anastasiou and Mitchell, 2013; Dufour and Le Bailly, 2013). This material was centrifuged and the supernatant removed leaving behind a small pellet, which was mixed with glycerol. Single drops were added to microscope slides to be viewed under a light microscope at 400× magnification. For each sample, five slides were viewed and, if any parasite eggs were identified, then the entire subsample was viewed to calculate eggs per gram. Parasite eggs were identified to the genus or species level based on morphological features (World Health Organization, 1994; Ash and Orihel, 2015; Garcia, 2016).

2.2.3. ELISA

ELISA has been used in paleoparasitology for at least two decades. ELISA kits designed to detect antigens in modern fecal samples have been shown to be effective at detecting ancient antigens as well (Le Bailly et al., 2016). Numerous labs have reported preserved protozoan antigens in archaeological samples (Allison et al., 1999; Le Bailly et al., 2008; Mitchell et al., 2008; Le Bailly and Bouchet, 2015; Graff et al., 2020). ELISA was not undertaken for all samples; pelvic sediment samples were not studied and only the samples from drains that contained helminth eggs (confirming the presence of fecal material in the samples) were studied. Thus, one drain sample (drain 5045) from Vagnari and two samples from the *cappuccina* drain (drain 931) at Vacone were tested.

ELISA tests made use of the sieved material from the catchment container below the 20 μm sieve; this is because cysts and oocysts from the protozoa tested for are typically smaller than 20 μm (Garcia, 2016). This material was centrifuged and the supernatant removed leaving behind a total volume of 2.5 mL to be used in each ELISA kit, one for each organism. The ELISA kits used for analysis were provided by TechLab (Blacksburg, USA). The manufacturer's protocols were followed, and one positive and negative control well were included on each test plate. The positive control was provided with the kits and was an antigen from the organism under investigation. An ELISA plate reader (BioTek Synergy HT Multi-Mode Microplate Reader) was used to generate absorbance values to determine which wells were positive by comparing them to reference values. A total of eight wells were used for each individual sample to act as replicative tests. Each test was also performed a second time on a separate date using a second subsample, thus forming a biological replication. Samples were considered positive if a positive well was found for both biological replicative tests. The kits have high sensitivity and specificity for modern samples (96.9–100 %) with no cross-reactivity for other common helminths and fecal organisms (Boone et al., 1999).

3. Results

3.1. Roman period

Microscopy revealed helminth eggs in samples from all Roman-period sites (Table 1 and Fig. 3; egg concentrations and measurements can be found in Appendix Table A.7). Pelvic sediment samples from Roman Imperial-period Italy came from four sites: Lucus Feroniae, Oplontis, Vacone, and Vagnari. In the samples from Lucus Feroniae, two of 14 individuals had *Ascaris* sp. (roundworm) eggs in the sediment collected from the sacrum. Eggs were found in sediment collected from the cranial bones of one of these individuals. As *Ascaris* sp. eggs should not be found in the cranium, this indicates that the burial environment was contaminated with eggs; however, the egg concentration was twice as high in the pelvis, making it more likely that this represents a true infection.

Ascaris lumbricoides (roundworm) and *Trichuris trichiura* (whipworm) were found in the pelvic sediment samples from Oplontis, in the absence

Table 1

Results of microscopy and ELISA for Roman Imperial-period and Longobard-period samples.

Site	Sample Type	Helminth Eggs	Protozoa
Roman Imperial Period			
Lucus Feroniae	Pelvic soil	*Ascaris* sp. (n = 2*/14)	Not tested
Oplontis	Pelvic soil	*Ascaris lumbricoides* (n = 2/8)	Not tested
		Trichuris trichiura (n = 1/8)	
Vacone	Drain fill	*Ascaris* sp.	*Giardia duodenalis*
		Trichuris trichiura	
	Pelvic soil	None (n = 0/1)	Not tested
	Drain fill	*Ascaris* sp.	*Giardia duodenalis*
Vagnari	Pelvic soil	*Trichuris trichiura* (n = 1/31)	
Late Antique and Longobard Period			
Povegliano Veronese	Pelvic soil	None (n = 0/14)	Not tested
Selvicciola	Pelvic soil	*Ascaris* sp. (n = 3*/15)	Not tested
		Taenia sp. (n = 2/15)	
Vacone	Pelvic soil	*Ascaris* sp. (n = 1/3)	Not tested

* Some control samples contained eggs.

of any eggs in the control samples. The high egg concentrations and absence of eggs in control samples indicate that these are true infections; thus the *Ascaris* eggs are most likely from the human-infecting species *Ascaris lumbricoides*. In one older child, *Ascaris lumbricoides* eggs were found, and in a young adult female both *Ascaris lumbricoides* and *Trichuris trichiura* eggs were found. The surface of many of the eggs appeared porous (Fig. 4), while typically modern decorticated *Ascaris* eggs have smooth shells, however the size fits within standard ranges for modern eggs (see Table A.7). Comparison with archaeobotanical and palynological work previously undertaken at the site confirmed that these differed from identified pollen and other non-pollen palynomorphs. It is possible that the irregular surface appearance is a result of the high temperatures generated by the eruption of Mount Vesuvius that ultimately resulted in the unique preservation conditions of these samples or were the result of acidic etching of the surface of the eggs from sulphur compounds contained within the volcanic ash.

At Vacone, *Ascaris* sp. eggs were found in the *cappuccina* drain along with a single *Trichuris trichiura* egg. Four samples were taken along the course of the drain and *Ascaris* sp. was found in all of the samples (140–620 epg). A single *Ascaris* egg was found in the sample from the possible latrine in room 33. No parasite eggs were found in the pelvic soil from the late Roman-period burials at the villa.

At Vagnari, *Trichuris trichiura* eggs were found in the pelvic sediment from one infant. *Ascaris* eggs were found in one of the Roman-period drain samples from Vagnari. Only one of the three drains tested contained parasite eggs. It is possible that the other two drains were used primarily for water runoff.

Giardia duodenalis antigen was detected in samples from two of the three Roman-period drain samples analysed using ELISA: one from Vagnari and one from Vacone. All samples were negative for *Entamoeba histolytica* and *Cryptosporidium* spp.

3.2. Late Antique and Longobard period

Helminth eggs were found in samples from the pelvic sediment of the Longobard-period individuals from Vacone, but none in the samples from Povegliano Veronese (Table 1 and Fig. 3). At Vacone, *Ascaris* sp. eggs were found in one of three individuals studied, an adult male. Unfortunately, no control samples could be collected from this burial for analysis.

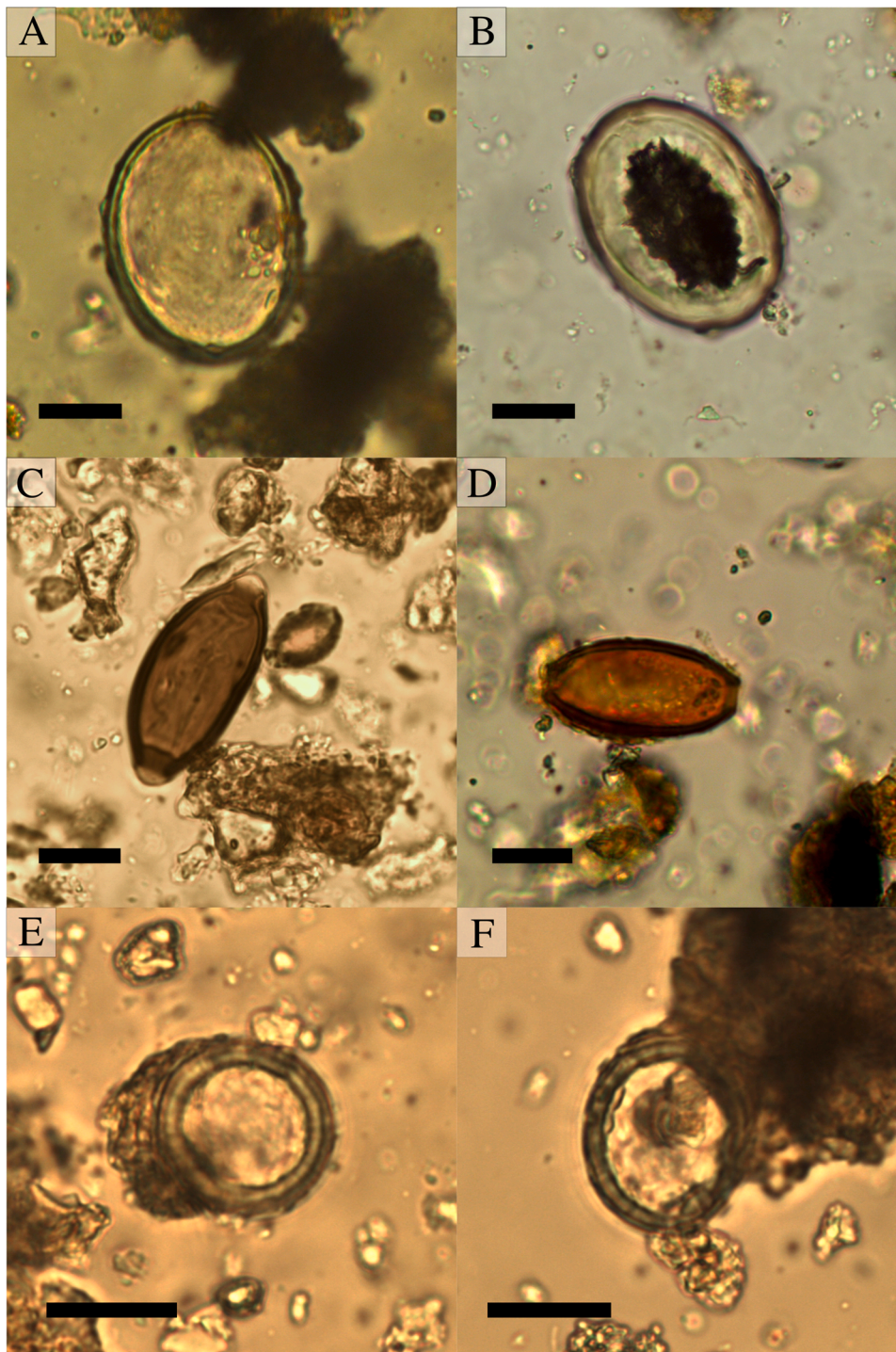Four of the fifteen individuals from Selvicciola had *Ascaris* sp. eggs in

Fig. 3. Helminth eggs identified in samples from Roman-period and Longobard-period sites. (A) decorticated *Ascaris* egg from a drain at Vacone (length: 61.6 μm; width: 47.8 μm); (B) decorticated *Ascaris* egg from Longobard-period pelvic sediment at Vacone (length: 61.4 μm; width: 45.5 μm); (C) *Trichuris trichiura* egg from Vagnari pelvic sediment (length: 58.0 μm; width: 25.6 μm); (D) *Trichuris trichiura* egg from Oplontis pelvic sediment (length: 47.7 μm; width: 23.8 μm); (E and F) *Taenia* sp. eggs from Selvicciola pelvic sediment (diameter 28.1 μm and 32.1 μm). Scale bars are 20 μm.

their pelvis. Two were likely buried in the Late Antique period prior to Longobard migrations and two are not dated to a specific period. One of the Late Antique-period individuals had only a single *Ascaris* egg in the pelvis and two eggs in the general burial sample, making it unlikely that this represents a true infection but rather contamination from the burial environment. Thus, one Late Antique individual and two others had evidence for infection. In the burial control samples from these three individuals, one or two *Ascaris* eggs were found. Since the location of the collected burial sediment samples was not recorded, they may not have originated from the area of the abdomen and pelvis where eggs would be found in an infected individual. These results need to be interpreted with caution, as they may not represent infections of these individuals.

However, they do confirm the presence of the parasites in the immediate burial area. Two of these individuals also had *Taenia* sp. (tapeworm) eggs in their pelvis; no *Taenia* eggs were found in the burial samples used as controls. As these individuals were not dated, they could have been buried at any time from the late 4th c. CE through to the early 8th c. CE.

4. Discussion

4.1. Consequences of parasite infection

Ascaris sp. (roundworm) and *Trichuris trichiura* (whipworm) both rely on the fecal-oral route for transmission; thus, their spread is
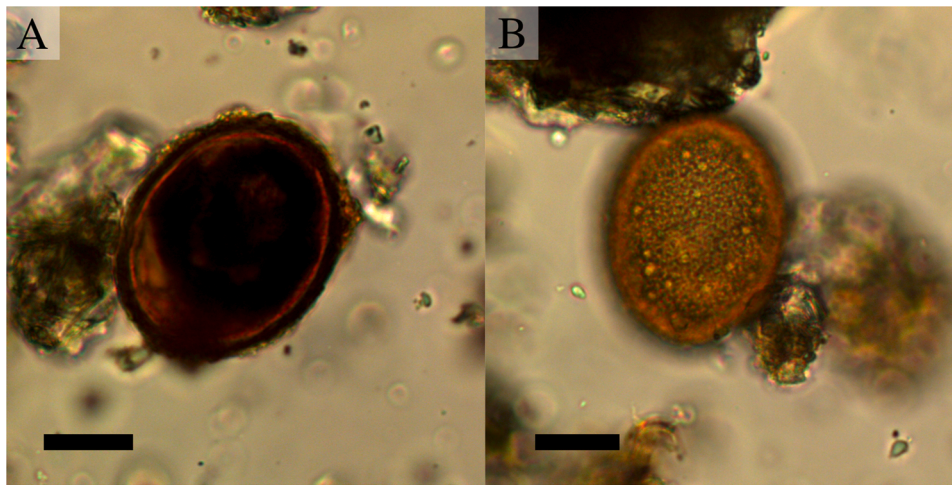
Fig. 4. Decorticated *Ascaris* eggs from Oplontis pelvic sediment samples. Dimensions of the egg in image A are 58.6 μm long and 47.9 μm wide. Surface porosity is visible in image B; dimensions are 56.8 μm long and 43.8 μm wide. Scale bars are 20 μm.

dependent upon hygiene and sanitation conditions (Strunz et al., 2014; Vaz Nery et al., 2019). Infection occurs after eggs are ingested; for example from contaminated food or water, from unwashed hands, or with plants grown in contaminated soil. Symptoms from roundworm and whipworm infection range considerably, with heavier worm burdens associated with higher morbidity (Bethony et al., 2006). Infections in children can cause growth impairment, impaired cognitive development, nutrient malabsorption, and intestinal obstruction in more severe cases (Bethony et al., 2006).

Giardia duodenalis is also transmitted by the fecal-oral route, often through ingestion of food or water contaminated with feces. Symptoms commonly include watery diarrhea, vomiting, weight loss, and abdominal pain (Wolfe, 1992). Similar to roundworm and whipworm, children have worse outcomes from infection, and some may die. In particular, chronic *Giardia* infection can result in failure to thrive (below average growth and weight gain). Some individuals are asymptomatic while others progress into a chronic carrier state after acute infection. *Giardia* can also cause long-term sequelae that affect numerous body organs (Robertson et al., 2010; Halliez and Buret, 2013).

Taenia sp. (beef/pork tapeworm) is a foodborne zoonotic disease (Ledger and Mitchell, 2019). Beef and pork tapeworms use humans as their definitive host, where the mature parasite is found, but they require either cows or pigs as intermediate hosts for parasite development. When humans are infected the adult worms are found in the intestines, and they release eggs in the feces. These eggs hatch, if ingested by cows or pigs, and larvae encyst within soft tissues such as muscle and visceral organs. When humans eat raw or undercooked meat from these animals, the larva develop into adult worms in the human host (Garcia, 2016). Thorough cooking of meat will kill parasite larvae and prevent infection (Smith, 1994). Thus, major risk factors for *Taenia* transmission are inadequate cooking of meat, disposal of human excrement in areas of animal grazing, and keeping free-ranging pigs (Hoberg, 2002). The eggs of *Taenia solium* (pork tapeworm) and *Taenia saginata* (beef tapeworm) are morphologically indistinguishable (Hoberg, 2002), so they are identified as *Taenia* sp. *T. solium* (pork tapeworm) has the unique ability to use humans as an accidental intermediate host. When humans ingest eggs, the larvae can encyst within their tissues, a disease known as cysticercosis. The central nervous system is the most common area in which larval cysts are found in humans, and this can cause numerous neurological symptoms such as seizures and focal neurological deficits, including visual impairment, limb weakness, and limb paralysis (Garcia et al., 2005).

4.2. Past analysis of parasites at Roman sites in Italy

Previous paleoparasitological analysis of samples from Roman-period sites in Italy have provided evidence for the helminths roundworm, whipworm, and *Fasciola* liver fluke, and the protozoan *Entamoeba histolytica*. Drain samples from Pompeii contained eggs of whipworm (Heirbaut et al., 2011). Sediment cores taken from Portus, one of the main ports for the city of Rome, contained eggs of roundworm, whipworm, and *Fasciola* sp. in layers dated between 25–660 CE (Dufour, 2015). However, due to the nature of the samples it is unclear if these eggs came from human or animal infections. Two sediment samples taken from a Roman-period core in Rome were tested for protozoa using ELISA, and *Entamoeba histolytica* was detected (Le Bailly and Bouchet, 2015). Finally, from a Late Antique cemetery in Florence (4th–5th c. CE) roundworm eggs were found in pelvic sediment samples from 5 of 18 individuals studied (Roche et al., 2019).

4.3. Interpreting the new data from this study

We have undertaken the most comprehensive multi-site paleoparasitological analysis of Roman Imperial period Italy and shown notable consistency between sites. Roundworm and whipworm were clearly common infections in these Roman sites. However, it appears that only a minority of individuals were infected at the time of their death, as more than 25 % positivity rates in the pelvic sediment of burials at any site was not found. This corresponds with previous data from 4th–5th century CE Florence, where 28 % of burials were positive for parasite eggs (Roche et al., 2019). The true prevalence of infection may have been higher since the parasite eggs originally present in some of those infected may have been lost due to the action of soil fungi, bacteria, and insects.

The ELISA results provide the first evidence for *Giardia duodenalis* in Roman-period Italy, at two separate sites. These results show that protozoa causing dysentery were transmitted in these communities alongside fecal-oral helminths. This complements previous results from ancient Roman samples collected from excavations in Rome where another parasite that causes dysentery, *Entamoeba histolytica*, was identified (Le Bailly and Bouchet, 2015). Together, this highlights how infective gastrointestinal diarrheal illness would have been a significant cause of ill health in Roman Italy.

In comparing these results with what is known about parasitic disease in the rest of the Roman Empire, the results from Italy match what has been found in other Mediterranean areas such as Turkey and Greece. In those regions, *Giardia*, roundworm, and whipworm have been

recovered in the near absence of foodborne zoonotic parasites (see Dufour, 2015 and Ledger et al., 2020 for a review of Roman-period studies). In sites that have been studied in regions of the Empire north of the Alps, such as in Britain and France, a more diverse range of parasite taxa has been recovered, including beef/pork tapeworm, *Capillaria*, fish tapeworm, and liver flukes (Pike, 1968; Rouffignac, 1985; Boyer, 1999; Bouchet et al., 2001b; Harter-Lailheugue, 2006; Le Bailly and Bouchet, 2010, 2013; Mowlavi et al., 2014).

This study also provides evidence for parasites in the Late Antique period in Italy just before and after Longobard migrations into Italy, which provides preliminary insight into disease transitions at the end of the Roman Empire in this region. The presence of roundworm infection in Late Antique and Longobard-period burials suggests that roundworm continued to infect people living in Italy at the end of the Roman Empire, whether they were ethnically Longobard or from local populations. However, we also found evidence for *Taenia* tapeworm in the pelvic samples from individuals from Selvicciola dating from the Late Antique period through the Longobard period, which was not found in any Imperial Roman samples. Other post-Roman evidence for parasites in Italy comes from later in the medieval period (10th–11th c. CE). In refuse pit sediments from Piazza Garibaldi in Parma, roundworm, *Taenia* or *Echinococcus* sp., and whipworm were found (Bosi et al., 2011; Florenzano et al., 2012). Potential explanations for the increase in zoonotic parasites in the Longobard and later medieval period include increased meat consumption, consumption of raw or preserved meats, changes to animal husbandry including fertilizing pastures with human fecal material, or keeping free-ranging pigs in areas where human excrement could contaminate the environment.

Previous paleoparasitological work has highlighted factors that may have contributed to the spread of sanitation-related parasites in the Mediterranean region during the Roman period (Mitchell, 2017; Williams et al., 2017; Ledger et al., 2018; Roche et al., 2019; Ledger et al., 2020). Much of this discussion draws on historical and archaeological studies of sanitation infrastructure in Roman Italy and is very much relevant for understanding parasite transmission in the communities studied here. A number of key areas where sanitation infrastructure design and use may have contributed to the spread of fecal-oral pathogens have been identified. Poor drainage or open cesspit designs would have been a risk. It has been suggested that there were large numbers of flies in Roman living spaces (Scobie, 1986) and analysis of latrine and sewer samples has confirmed their presence in some sites (Knights et al., 1983; Rowan, 2014). Flies are common vectors for spreading parasite eggs (Maipanich et al., 2008), and the location of many private latrines in kitchens (Pérez et al., 2011) means these eggs could easily be transferred from open latrines to food preparation areas or food itself. Where latrines were not used, chamber pots were a common alternative, and these would have been emptied into sewers, latrines, or on dung heaps. These were practices that could easily contaminate cities and living spaces and bring some individuals into close contact with the excrement of others (Taylor, 2015; Trusler and Hobson, 2017). There is some evidence for open defecation in Roman cities (Wilson, 2000), a practice strongly linked to the spread of soil-transmitted helminths (Grimes et al., 2016; Muluneh et al., 2020). Sharing multi-seat latrines, the reuse of latrine sticks for anal washing after using the latrine, and hand washing at a time when soap was generally not used when washing hands, could all have spread parasites and other gastrointestinal pathogens.

The results of this study provide important insights into the burden of gastrointestinal disease in Roman-period Italy. The presence of multiple species of parasites transmitted by the fecal-oral route in sites across Roman Italy suggests that gastrointestinal infections were fairly common. Viruses and bacteria that are transmitted by the fecal-oral route would have been able to flourish in these communities, alongside helminths and protozoa. Modern epidemiological studies show that children carry a large burden of disease both in terms of prevalence of infections and symptoms from infection. The presence of whipworm infection in an infant from Vagnari and roundworm in children from

Oplontis and Selvicciola indicates that young individuals were likely at high risk for infection in these communities as well. Some individuals studied (n = 3/73; 4 %) had eggs from two different taxa in their pelvis, indicating co-infections with these parasites. From Oplontis, one individual had roundworm and whipworm eggs in their pelvis, and from Selvicciola two individuals had roundworm and beef/pork tapeworm eggs. Although tests for *Giardia* in the pelvic sediment samples was not undertaken, it is possible that some individuals had helminth-protozoa co-infections as well. Polyparasitism is common in endemic regions today (Donohue et al., 2019), and the fact that it was found in a relatively small sample size indicates it was probably common in the Roman period as well. Polyparasitism is sometimes linked to higher morbidity than monoparasitism (Nematian et al., 2008; Pullan and Brooker, 2008; Donohue et al., 2019).

As with all research, there are some limitations to this study. Ideal control samples were not available for all of the Late Antique and Longobard-period samples. This, in conjunction with the presence of parasite eggs in some of the general burial samples (see Appendix), means some of the results should be interpreted with caution, as eggs in the pelvic area may represent contamination of the burial environment rather than true infection. Regardless, the recovery of parasite eggs would indicate the presence of these parasites in the population at that time in order for the soil to become contaminated with eggs. When working with pelvic sediment samples, only the infections that were present at the time of death are identifiable. Children between 5 and 15 years of age are the most commonly infected age group with soil-transmitted helminths (Hotez et al., 2006). Children who survive past the age of five often have higher life expectancy in modern populations, which would limit our ability to detect parasite infections in pelvic soil samples. This would be the case if infected individuals survived these high-risk years and died in later adulthood when they were no longer infected. One exception to this is the samples from Oplontis, which are from a catastrophic event providing information about disease at the same time point for all individuals excavated. In addition, helminths are often over-dispersed in modern populations (Churcher et al., 2005), with a small percentage of a population harboring most worms. Poor preservation of eggs, and the fact that only a small proportion of individuals from any one community were studied, may mean low intensity infections were not detected, and those individuals with the highest worm burdens are most likely to be identified. However, the study of latrine and sewer samples alongside pelvic soil samples increases the chances of detecting infections, as these latrine and sewer samples would contain concentrated fecal material from numerous individuals collected over the entire period of use (or use since last drainage or emptying), rather than just at the time of death. Despite these limitations, this study contributes additional evidence for parasitic infection in Roman-period Italy, doubling the number of sites studied to date. This study also provides the first evidence for *Giardia duodenalis* in Roman communities in Italy and is the first paleoparasitological analysis of Longobard-period samples of which we are aware.

5. Conclusions

Paleoparasitological analysis of four sites from Roman Italy, coupled with data from four sites previously studied, has shown that fecal-oral parasites were widespread in Imperial Roman Italy. Parasites found in Roman samples from Italy include the helminths *Ascaris* and *Trichuris* and protozoa that can cause dysentery, namely *Entamoeba histolytica* and *Giardia duodenalis*. There does not appear to be any difference in the types of parasites found in urban centers such as Rome compared to countryside villas like Vacone or rural estates like Vagnari. The widespread presence of these parasites and factors allowing for their spread suggest that gastrointestinal infections, in general, would have caused a significant burden of disease in Roman Italy, particularly in children. Co-infections by more than one species were likely present, as are found in endemic regions today. Additionally, the analysis of samples from the

transitional period from Late Antiquity through the Longobard-period shows continuity in the transmission of sanitation-related parasites, but also the appearance of zoonotic foodborne parasites acquired from eating undercooked meat, which have not been previously found in Roman Italy.

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Appendix A. Supplementary data

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References

Allison, M.J., Bergman, T., Gerszten, E., 1999. Further studies on fecal parasites in antiquity. Am. J. Clin. Pathol. 112, 605–609.

Alt, W.K., Knipper, C., Peters, D., Müller, W., Maurer, A.F., Kollig, I., Nicklisch, N., Müller, C., Karimnia, S., Brandt, G., Roth, C., Rosner, M., Mende, B., Schöne, B.R., Vida, T., von Freeden, U., 2014. Lombards on the move–an integrative study of the Migration Period cemetery at Szólád, Hungary. PLoS One 9 (11), e110793.

Amorim, C.E.G., Vai, S., Posth, C., Modi, A., Koncz, I., Hakenbeck, S., La Rocca, M.C., Mende, B., Bobo, D., Pohl, W., Baricco, L.P., Bedini, E., Francalacci, P., Giostra, C., Vida, T., Winger, D., von Freeden, U., Ghirotto, S., Lari, M., Barbujani, G., Krause, J., Caramelli, D., Geary, P.J., Veeramah, K.R., 2018. Understanding 6th-century barbarian social organization and migration through paleogenomics. Nat. Commun. 9 (1), 3547.

Anastasiou, E., Mitchell, P.D., 2013. Simplifying the process of extracting intestinal parasite eggs from archaeological sediment samples: a comparative study of the efficacy of widely-used disaggregation techniques. Int. J. Paleopathol. 3 (3), 204–207.

Argenti, M., Manzi, G., 1988. Morfometria cranica delle popolazioni romani di età imperiale: Isola Sacra e Lucus Feroniae. Rivista di Antropologia 66, 179–200.

Ash, L.R., Orihel, T.C., 2015. Atlas of Human Parasitology, fifth ed. American Society for Clinical Pathology Press, Chicago.

Aspöck, H., Feuereis, I., Radbauer, S., 2011. Case study: detection of eggs of the intestinal parasite *Ascaris lumbricoides* in samples from the Roman sewers of Carnuntum. In: Jansen, G.C.M., Koloski-Ostrow, A.O., Moormann, E.M. (Eds.), Roman Toilets: Their Archaeology and Cultural History. Peeters, Leuven, pp. 163–164.

Bethony, J., Brooker, S., Albonico, M., Geiger, S.M., Loukas, A., Diemert, D., Hotez, P.J., 2006. Soil-transmitted helminth infections: ascariasis, trichuriasis, and hookworm. Lancet 367 (9521), 1521–1532.

Boone, J.H., Wilkins, T.D., Nash, T.E., Brandon, J.E., Macias, E.A., Jerris, R.C., Lyerly, D. M., 1999. TechLab and alexon *Giardia* enzyme-linked immunosorbent assay kits detect cyst wall protein 1. J. Clin. Microbiol. 37 (3), 611–614.

Bosi, G., Mazzanti, M.B., Florenzano, A., N`siala, I.M., Pederzoli, A., Rinaldi, R., Torri, P., Mercuri, A.M., 2011. Seeds/fruits, pollen and parasite remains as evidence of site function: piazza Garibaldi – Parma (N Italy) in Roman and Mediaeval times. J. Archaeol. Sci. 38 (7), 1621–1633.

Bouchet, F., West, D., Lefèvre, C., Corbett, D., 2001a. Identification of parasitoses in a child burial from Adak Island (Central Aleutian Islands, Alaska). Comptes Rendus de l'Académie des Sciences. Serie III, Sciences de la Vie 324 (2), 123–127.

Bouchet, F., Bentrad, S., Martin, C., 2001b. Le quartier Gallo-romain de la rue de Venise à Reims, Étude paléoparasitologique. Bulletin de la Société Archéologique Champenoise 2/3, 148–150.

Boyer, P., 1999. The parasites. In: Connor, A., Buckley, R. (Eds.), Roman and Medieval Occupation of Causeway Lane, Leicester Excavations 1980 and 1991. University of Leicester Archaeological Services, Leicester, pp. 344–346.

Bruno, B., Giostra, C., 2012. Il territorio di Povegliano Veronese fra tarda antichità e alto medioevo: nuovi dati e prime riflessioni. In: Redi, F., Forgione, A. (Eds.), VI Congresso Nazionale di Archeologia Medievale, L'Aquila, 12–15 settembre 2012. All'Insegna del Giglio, Firenze, pp. 216–222.

Carroll, M., 2019. Preliminary Report on the University of Sheffield Excavations in the *Vicus* of the Roman Imperial Estate at Vagnari, Puglia, 2012-2018. FastiOnline FOLD&R, 2019-431. www.fastionline.org/docs/FOLDER-it-2019-431.pdf.

Churcher, T.S., Ferguson, N.M., Basáñez, M.G., 2005. Density dependence and overdispersion in the transmission of helminth parasites. Parasitology. 131 (Pt 1), 121–132.

Cooley, A.E., 2016. A Companion to Roman Italy. Wiley Blackwell, Chichester.

Dittmar, K., Teegen, W.-R., Cordie-Hackenberg, R., 2002. Nachweis von Eingeweideparasiteneiern in einem Abfallschacht aus dem römischen Vicus von Belginum/Wederath (Rheinland-Pfalz). Archäologisches Korrespondenzblatt 32, 415–425.

Donohue, R.E., Cross, Z.K., Michael, E., 2019. The extent, nature, and pathogenic consequences of helminth polyparasitism in humans: a meta-analysis. PLoS Negl. Trop. Dis. 13 (6), e0007455.

Dufour, B., 2015. Synthèse de données et nouvelle contribution à l'étude des parasites de l'époque romaine, et apports méthodologiques de l`extraction des marqueurs au traitement des résultats. PhD. Université de Franche-Comte.

Dufour, B., Le Bailly, M., 2013. Testing new parasite egg extraction methods in paleoparasitology and an attempt at quantification. Int. J. Paleopathol. 3 (3), 199–203.

Emery, M.V., Duggan, A.T., Murchie, T.J., Stark, R.J., Klunk, J., Hider, J., Eaton, K., Karpinski, E., Schwarcz, H.P., Poinar, H.N., Prowse, T.L., 2018a. Ancient Roman mitochondrial genomes and isotopes reveal relationships and geographic origins at the local and pan-Mediterranean scales. J. Archaeol. Sci. Rep. 20, 200–209.

Emery, M.V., Stark, R.J., Murchie, T.J., Elford, S., Schwarcz, H.P., Prowse, T.L., 2018b. Mapping the origins of Imperial Roman workers (1st-4th century CE) at Vagnari, Southern Italy, using 87 Sr/86 Sr and δ18 O variability. Am. J. Phys. Anthropol. 166 (4), 837–850.

Fletcher, S.M., McLaws, M.-L., Ellis, J.T., 2013. Prevalence of gastrointestinal pathogens in developed and developing countries: systematic review and meta-analysis. J. Public Health Res. 2 (1), 42–53.

Florenzano, A., Mercuri, A.M., Pederzoli, A., Torri, P., Bosi, G., Olmi, L., Rinaldi, R., Mazzanti, M.B., 2012. The significance of intestinal parasite remains in pollen samples from Medieval pits in the Piazza Garibaldi of Parma, Emilia Romagna, Northern Italy. Geoarchaeology 27, 34–47.

Francisci, G., Micarelli, I., Iacumin, P., Castorina, F., Di Vincenzo, F., Di Matteo, M., Giostra, C., Manzi, G., Tafuri, M.A., 2020. Strontium and oxygen isotopes as indicators of Longobards mobility in Italy: an investigation at Povegliano Veronese. Sci. Rep. 10 (1), 1–12.

Franconi, T.V., Rice, C., Bloy, D., Farney, G., 2019. Excavations at the Roman villa of Vacone (Lazio) by the Upper Sabina Tiberina Project, 2012-2018. In: Sfameni, C., Volpi, M. (Eds.), Oltre la Villa: Richerche nei siti archeologici del territorio di Cottanello, Configni, Vacone e Montasola. Arbor Sapientiae, Rome, pp. 101–128.

Garcia, L.S., 2016. Diagnostic Medical Parasitology. ASM Press, Washington, DC.

Garcia, H.H., Del Brutto, O.H., Cysticercosis Working Group in Peru, 2005. Neurocysticercosis: updated concepts about an old disease. Lancet Neurol. 4 (10), 653–661.

Garnsey, P., Saller, R., 2015. The Roman Empire: Economy, Society and Culture, second ed. University of California Press, Berkeley.

Gazzetti, G., 1985. La villa rustica della Selvicciola: risultati delle prime quattro campagne di scavo. Archeologia 85, 9–16.

Gazzetti, G., 1986. La colonia romana di Lucus Feroniae e suburbia. In: Michele, Ospiziodi San (Ed.), Tevere: Un'Antica Via per il Mediterraneo. Istituto Poligrafico dello Stato, Roma, pp. 193–194.

Global Burden of Disease 2016 Diarrhoeal Disease Collaborators, 2016. Estimates of the global, regional, and national morbidity, mortality, and aetiologies of diarrhoea in 195 countries: a systematic analysis for the Global Burden of Disease Study 2016. Lancet Infect. Dis. 18 (11), 1211–1228.

Giostra, C., 2014. La necropoli di Povegliano Veronese, loc. Ortaia. In: Possenti, E. (Ed.), Necropoli Longobarde in Italia: Indirizzi della Ricerca e Nuovi Dati. Castello del Buonconsiglio, Trento, pp. 239–272.

Goffart, W.A., 2006. Barbarian Tides: the Migration Age and the Later Roman Empire. University of Pennsylvania Press, Philadelphia.

Graff, A., Bennion-Pedley, E., Jones, A.K., Ledger, M.L., Deforce, K., Degraeve, A., Byl, S., Mitchell, P.D., 2020. A comparative study of parasites in three latrines from Medieval and Renaissance Brussels, Belgium (14th-17th centuries). Parasitology 147 (13), 1443–1451.

Grimes, J.E.T., Tadesse, G., Mekete, K., Wuletaw, Y., Gebretsadik, A., French, M.D., Harrison, W.E., Drake, L.J., Gardiner, I.A., Yard, E., Templeton, M.R., 2016. School water, sanitation, and hygiene, soil-transmitted helminths, and schistosomes: national mapping in Ethiopia. PLoS Negl. Trop. Dis. 10 (3), e0004515.

Halliez, M.C.M., Buret, A.G., 2013. Extra-intestinal and long term consequences of *Giardia duodenalis* infections. World J. Gastroenterol. 19 (47), 8974–8985.

Harter-Lailheugue, S., 2006. Étude paléoparasitologique du site de Marseille Quai Rive Neuve. In: Bien, S., Richier, A. (Eds.), Rapport final d'Opération, Fouilles Archéologiques, 23, Quai de Rive Neuve à Marseille (Bouches-du-Rhône). Inrap Méditerranée, pp. 91–95.

Heirbaut, E., Jones, A.K.G., Wheeler, K., 2011. Archeaeometry: methods and analysis. In: Jansen, G.C.M., Koloski-Ostrow, A.O., Moormann, E.M. (Eds.), Roman Toilets: Their Archaeology and Cultural History. Peeters, Leuven, pp. 7–20.

Hoberg, E.P., 2002. *Taenia* tapeworms: their biology, evolution and socioeconomic significance. Microbes Infect. 4 (8), 859–866.

Hotez, P.J., Bundy, D.A.P., Beegle, K., Brooker, S., Drake, L., de Silva, N., Montresor, A., Engels, D., Jukes, M., Chitsulo, L., Chow, J., Laxminarayan, R., Michaud, C., Bethony, J., Correa-Oliveira, R., Shuhua, X., Fenwick, A., Savioli, L., 2006. Helminth infections: soil-transmitted helminth infections and schistosomiasis. In: Jamison, D. T., Breman, J.G., Measham, A.R., Alleyne, G., Claeson, M., Evans, D.B., Jha, P., Mills, A., Musgrove, P. (Eds.), Disease Control Priorities in Developing Countries. Oxford University Press, New York.

Keay, S., Terrenato, N., 2001. Italy and The West: Comparative Issues in Romanization. Oxbow, Oxford.

Killgrove, K., 2018. Oplontis Skeletal Project 2017-2018: Risultati dello scavo e analisi. Relazione scientific preliminare (Dicembre 2018). Report to Giuseppe Scarpati, Parco Archeologico di Pompei, 15 pp.

Knights, B.A., Dickson, C.A., Dickson, J.H., Breeze, D.J., 1983. Evidence concerning the Roman military diet at Bearsden, Scotland, in the 2nd Century AD. J. Archaeol. Sci. 10 (2), 139–152.

Le Bailly, M., Bouchet, F., 2010. Ancient dicrocoeliosis: occurrence, distribution and migration. Acta Trop. 115 (3), 175–180.

Le Bailly, M., Bouchet, F., 2013. *Diphyllobothrium* in the past: review and new records. Int. J. Paleopathol. 3 (3), 182–187.

Le Bailly, M., Bouchet, F., 2015. A first attempt to retrace the history of dysentery caused by *Entamoeba histolytica*. In: Mitchell, P.D. (Ed.), Sanitation, Latrines and Intestinal Parasites in Past Populations. Ashgate, Farnham, pp. 219–228.

Le Bailly, M., Gonçalves, M.L., Harter-Lailheugue, S., Prodéo, F., Araújo, A., Bouchet, F., 2008. New finding of *Giardia intestinalis* (Eukaryote, Metamonad) in Old World archaeological site using immunofluorescence and enzyme-linked immunosorbent assays. Memorias do Instituto Oswaldo Cruz 103 (3), 298–300.

Le Bailly, M., Maicher, C., Dufour, B., 2016. Archaeological occurrences and historical review of the human amoeba, *Entamoeba histolytica*, over the past 6000 years. Infect. Genet. Evol. 42, 34–40.

Ledger, M.L., Mitchell, P.D., 2019. Tracing zoonotic parasite infections throughout human evolution. Int. J. Osteoarchaeol. 1–12. https://doi.org/10.1002/oa.2786.

Ledger, M.L., Stock, F., Schwaiger, H., Knipping, M., Brückner, H., Ladstätter, S., Mitchell, P.D., 2018. Intestinal parasites from public and private latrines and the harbour canal in Roman Period Ephesus, Turkey (1st c. BCE to 6th c. CE). J. Archaeol. Sci. Rep. 21, 289–297.

Ledger, M.L., Rowan, E., Marques, F.G., Sigmier, J.H., Šarkić, N., Redžić, S., Cahill, N.D., Mitchell, P.D., 2020. Intestinal parasitic infection in the Eastern Roman Empire during the Imperial Period and Late Antiquity. Am. J. Archaeol. 124 (4), 631–657.

Maipanich, W., Sanguankiat, S., Pubampen, S., Kusolsuk, T., Rojekittikhun, W., Castelli, F., 2008. House flies: potential transmitters of soil-transmitted-helminth infections in an unsanitary community. J. Trop. Med. Parasitol. 31, 14–22.

Manzi, G., Censi, L., Sperduti, A., Passarello, P., 1989. Linee di Harris e ipoplasia dello smalto nei resti scheletrici delle popolazioni umane di Isola Sacra e Lucus Feroniae (Roma I-III sec. d.C.). Rivista di Antropologia 67, 129–148.

Manzi, G., Santandrea, E., Passarello, P., 1997. Dental size and shape in the Roman Imperial age: two examples from the area of Rome. Am. J. Phys. Anthropol. 102 (4), 469–479.

Manzi, G., Salvadei, L., Vienna, A., Passarello, P., 1999. Discontinuity of life conditions at the transition from the Roman imperial age to the early middle ages: example from central Italy evaluated by pathological dento-alveolar lesions. Am. J. Hum. Biol. 11 (3), 327–341.

Micarelli, I., 2020. All'origine dei mestieri: attività professionali e strutture sociali in comunità alto-medievali in Italia. Un'indagine bioarcheologica applicata a due necropoli di cultura longobarda. Ph.D. Dissertation. Sapienza University of Rome.

Micarelli, I., Paine, R., Giostra, C., Tafuri, M.A., Profico, A., Boggioni, M., Di Vincenzo, F., Massani, D., Papini, A., Manzi, G., 2018. Survival to amputation in pre-antibiotic era: a case study from a Longobard necropolis (6th-8th centuries AD). J. Anthropol. Sci. 96, 185–200.

Micarelli, I., Paine, R.R., Tafuri, M.A., Manzi, G., 2019. A possible case of mycosis in a post-classical burial from La Selvicciola (Italy). Int. J. Paleopathol. 24, 25–33.

Mitchell, P.D. (Ed.), 2015. Sanitation, Latrines and Intestinal Parasites in Past Populations. Ashgate, Farnham.

Mitchell, P.D., 2017. Human parasites in the Roman World: health consequences of conquering an empire. Parasitology 144 (1), 48–58.

Mitchell, P.D., Stern, E., Tepper, Y., 2008. Dysentery in the crusader kingdom of Jerusalem: an ELISA analysis of two medieval latrines in the City of Acre (Israel). J. Archaeol. Sci. 35 (7), 1849–1853.

Mowlavi, G., Kacki, S., Dupouy-Camet, J., Mobedi, I., Makki, M., Harandi, M.F., Naddaf, S.R., 2014. Probable hepatic capillariosis and hydatidosis in an adolescent from the late Roman period buried in Amiens (France). Parasite 21, 9.

Muluneh, C., Hailu, T., Alemu, G., 2020. Prevalence and associated factors of soil-transmitted helminth infections among children living with and without open defecation practices in Northwest Ethiopia: a comparative cross-sectional study. Am. J. Trop. Med. Hyg. 103 (1), 266–272.

Muslin, J.L., 2016. Working and living in Oplontis B: material perspectives on trade and consumption. In: Gazda, E.K., Clarke, J.R. (Eds.), Leisure and Luxury in the Age of

Nero: The Villas of Oplontis Near Pompeii. Kelsey Museum of Archaeology, Ann Arbor, pp. 166–170.

Nematian, J., Gholamrezanezhad, A., Nematian, E., 2008. Giardiasis and other intestinal parasitic infections in relation to anthropometric indicators of malnutrition: a large, population-based survey of schoolchildren in Tehran. Ann. Trop. Med. Parasitol. 102 (3), 209–214.

Patterson, H., Witcher, R., Di Giuseppe, H., 2020. The Changing Landscapes of Rome's Northern Hinterland: The British School at Rome's Tiber Valley Project. Archaeopress, Oxford.

Pérez, J.A., Flohr, M., Hobson, B., Koehler, J., Koloski-Ostrow, A.O., Radbauer, S., Vaerenbergh, J.V., 2011. Location and contexts of toilets. In: Jansen, G.C.M., Koloski-Ostrow, A.O., Moormann, E.M. (Eds.), Roman Toilets: Their Archaeology and Cultural History. Peeters, Leuven, pp. 113–131.

Petznek, B., 2018. A Roman cesspit from the mid-2nd century with lead price tags in the civil town of Carnuntum (Schloss Petronell/Austria). In: Hoss, S. (Ed.), Latrinae: Roman Toilets in the Northwestern Provinces of the Roman Empire. Archaeopress, Oxford, pp. 119–126.

Pike, A.W., 1968. Recovery of helminth eggs from archaeological excavations, and their possible usefulness in providing evidence for the purpose of an occupation. Nature 219 (5151), 303–304.

Prowse, T., Carroll, M., 2017. Exploring the Roman Imperial Estate at Vagnari, Puglia (Commune di Gravina in Puglia, Provincia di Bari, Regione Puglia). Pap. Br. Sch. Rome 85, 330–334.

Prowse, T.L., Barta, J.L., von Hunnius, T.E., Small, A.M., 2010. Stable isotope and ancient DNA evidence for geographic origins at the site of Vagnari (2nd- 4th centuries AD), Italy. In: Eckhart, H. (Ed.), Roman Diasporas: Archaeological Approaches to Mobility and Diversity in the Roman Empire. Journal of Roman Archaeology Supplement, 78, pp. 175–198.

Pullan, R., Brooker, S., 2008. The health impact of polyparasitism in humans: are we under-estimating the burden of parasitic diseases? Parasitology 135 (7), 783–794.

Robertson, L.J., Hanevik, K., Escobedo, A.A., Mørch, K., Langeland, N., 2010. Giardiasis–why do the symptoms sometimes never stop? Trends Parasitol. 26 (2), 75–82.

Roche, K., Pacciani, E., Bianucci, R., Le Bailly, M., 2019. Assessing the parasitic burden in a Late Antique Florentine emergency burial site. Korean J. Parasitol. 57 (6), 587–593.

Rouffignac, C., 1985. Parasite egg survival and identification from Hibernia Wharf, Southwark. Lond. Archaeol. 5 (4), 103.

Rowan, E., 2014. Roman Diet and Nutrition in the Vesuvian Region: a Study of the Bioarchaeological Remains from the Cardo V Sewer at Herculaneum. DPhil. University of Oxford.

Salvadei, L., Santandrea, E., Manzi, G., Passarello, P., 1995. I Longobardi di La Selvicciola (Ischia di Castro, Viterbo). III—Morfologia e morfometria dentaria. Rivista di Antropologia 73, 281–290.

Salvadei, L., Ricci, F., Manzi, G., 2001. Porotic hyperostosis as a marker of health and nutritional conditions during childhood: studies at the transition between Imperial Rome and the Early Middle Ages. Am. J. Hum. Biol. 13 (6), 709–717.

Scobie, A., 1986. Slums, sanitation, and mortality in the Roman world. KLIO 68, 399–433.

Scopacasa, R., 2016. Rome's encroachment on Italy. In: Cooley, A.E. (Ed.), A Companion to Roman Italy. Wiley Blackwell, Hoboken, NJ, pp. 75–101.

Small, A.M., 2011. Vagnari: Il Villaggio, l'Artigianato, la Proprietà Imperiale. Edipuglia, Bari.

Smith, J.L., 1994. *Taenia solium* neurocysticercosis. J. Food Prot. 57 (9), 831–844.

Sperduti, A., Manzi, G., Salvadei, L., Passarello, P., 1995. I Longobardi di La Selvicciola (Ischia di Castro, Viterbo). II—Morfologia e morfometria scheletrica. J. Anthropol. Sci. 73, 265–279.

Strunz, E.C., Addiss, D.G., Stocks, M.E., Ogden, S., Utzinger, J., Freeman, M.C., 2014. Water, sanitation, hygiene, and soil-transmitted helminth infection: a systematic review and meta-analysis. PLoS Med. 11 (3), e1001620.

Tafuri, M.A., Goude, G., Manzi, G., 2018. Isotopic evidence of diet variation at the transition between classical and post-classical times in Central Italy. J. Archaeol. Sci. Rep. 21, 496–503.

Taylor, C., 2015. A tale of two cities: the efficacy of ancient and medieval sanitation methods. In: Mitchell, P.D. (Ed.), Sanitation, Latrines and Intestinal Parasites in Past Populations. Ashgate, Farnham, pp. 69–97.

Thomas, M.L., 2016. Oplontis B and the wine industry in the Vesuvian area. In: Gazda, E. K., Clarke, J.R. (Eds.), Leisure and Luxury in the Age of Nero: The Villas of Oplontis Near Pompeii. Kelsey Museum of Archaeology, Ann Arbor, MI, pp. 160–165.

Trusler, A.K., Hobson, B., 2017. Downpipes and upper story latrines in Pompeii. J. Archaeol. Sci. Rep. 13, 652–665.

van der Graaff, I., Muslin, J.L., Thomas, M.L., Wilkinson, P., Clarke, J.R., Muntasser, N. K., 2016. Preliminary Notes on Two Seasons of Research at Oplontis B (2014-2015). Fasti Online Documents & Research, p. 362.

Vaz Nery, S., Pickering, A.J., Abate, E., Asmare, A., Barrett, L., Benjamin-Chung, J., Bundy, D.A.P., Clasen, T., Clements, A.C.A., Colford Jr., J.M., Ercumen, A., Crowley, S., Cumming, O., Freeman, M.C., Haque, R., Mengistu, B., Oswald, W.E., Pullan, R.L., Oliveira, R.G., Einterz Owen, K., Walson, J.L., Youya, A., Brooker, S.J., 2019. The role of water, sanitation and hygiene interventions in reducing soil-transmitted helminths: interpreting the evidence and identifying next steps. Parasit. Vectors 12 (273), 1–8.

Williams, F.S., Arnold-Foster, T., Yeh, H.-Y., Ledger, M.L., Baeten, J., Poblome, J., Mitchell, P.D., 2017. Intestinal parasites from the 2nd-5th century AD latrine in the Roman Baths at Sagalassos (Turkey). Int. J. Paleopathol. 19, 37–42.

Wilson, A., 2000. Incurring the wrath of mars: sanitation and hygiene in Roman North Africa. In: Jansen, G. (Ed.), *Cura Aquarum* in Sicilia: Proceedings of the Tenth

International Congress on the History of Water Management and Hydraulic Engineering in the Mediterranean Region. Peeters, Leiden, pp. 307–312.

Wolfe, M.F., 1992. Giardiasis. Clin. Microbiol. Rev. 5, 93–100.

Woolf, G., 1997. Beyond romans and natives. World Archaeol. 28 (3), 339–350.

World Health Organization, 1994. Bench Aids for the Diagnosis of Intestinal Parasites. World Health Organization, Geneva.