Intestinal parasites in the Roman Empire, their regional distribution and ecosocial determinants

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Declaration

This thesis is the result of my own work and includes nothing which is the outcome of work done in collaboration except as declared in the Preface and specified in the text. It is not substantially the same as any that I have submitted, or, is being concurrently submitted for a degree or diploma or other qualification at the University of Cambridge or any other University or similar institution except as declared in the Preface and specified in the text. I further state that no substantial part of my thesis has already been submitted, or, is being concurrently submitted for any such degree, diploma or other qualification at the University of Cambridge or any other University or similar institution except as declared in the Preface and specified in the text. It does not exceed the prescribed word limit for the relevant Degree Committee.

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Abstract

As one of the main categories of pathogens, an understanding of parasitic infection in the past is essential to understanding disease in past populations. Evidence for parasites in the human past is steadily growing with examples from many different continents and time periods. Studies have been done in the Roman period however, this evidence does not cover all regions of the Roman empire. At its greatest extent the empire covered much of Europe, North Africa, and parts of the Middle East and different regions had strong cultural influences, not only from pre-existing cultural groups, but also from connections outside the empire. For these reasons there are likely to be variations in disease presence regionally. One of the main aims of this dissertation is to illuminate possible regional variations in parasitic infection in the Roman empire and compare this to earlier and later time periods in order to consider possible explanations for parasite taxonomic diversity in the empire. Archaeological sediments were collected from Roman period sites in previously understudied regions, such as Italy, Turkey, and the frontier regions, as well as pre-Roman and post-Roman time periods that had limited or no data. These samples were analysed for preserved parasite eggs and cysts using microscopy and enzyme-linked immunosorbent assay (ELISA). In the Roman period, predominately soil-transmitted helminths were found in samples from the Mediterranean region while those from northern Europe had additional zoonotic taxa. In the pre-Roman period the taxonomic diversity found was much higher, especially at the Bronze Age site of Must Farm in the marshes of Britain. The parasites found in the post-Roman sites studied here were very similar to that in the Roman period. A consideration of sanitation infrastructure presence, design, and use based on archaeological and historical evidence points to some potential reasons for the consistent presence of roundworm and whipworm across the Roman empire. Additionally, differences in diet, cooking practices, animal husbandry, and climate are considered as contributors to taxonomic diversity in different regions of the empire.

Preface

This thesis has been completed under the supervision of Dr. Piers Mitchell in the Department of Archaeology beginning in October 2016. This thesis is the result of my own work and to the best of my ability I have indicated throughout where material was contributed by collaborators, in most cases this was to provide context for the materials being studied. Some parts of this thesis have already been published or submitted for publication and I have detailed those below and have tried to clearly state this throughout the text. Copies of that material can be found in Appendix A and a full list of publications in Section 10.

In section 2 the overview of the evolution of parasites formed the start of a publication in the *International Journal of Osteoarchaeology* (Ledger and Mitchell, 2019). This publication was written by me with Dr. Mitchell as supervising author.

The work on Must Farm in section 4.2.1 has been published in *Parasitology* (Ledger et al., 2019a). Some of the sediment samples collected from the site were analysed by other students in our lab, Madison Fairey and Elisabeth Grimshaw, the details of which samples were analysed by me is described in the materials section. All parasites identified were found in the samples that I analysed and many were found in the samples they analysed as well. This manuscript was written by me with edits and input from collaborators. The section on lipid biomarkers was written by Helen Whelton and Ian Bull who completed this analysis. Dr. Mitchell was the supervising author of this publication.

The work on Çatalhöyük in section 4.2.2 has been published in *Antiquity* (Ledger et al., 2019b). The work of this thesis was combined with analysis of separate samples from the site by Dr. Evilena Anastasiou in that publication. The material described in this thesis is only the samples that I studied. This publication was written jointly by myself and Dr. Mitchell with input from all coauthors.

The work on Viminacium in section 4.3.9 has been incorporated into a manuscript submitted for publication in the *American Journal of Archaeology* (Appendix A.5). This article was written by me and all analysis described was completed by me. Dr. Mitchell was the supervising author and all coauthors also gave their input.

The work on Ephesus in section 4.3.10 has been published in the *Journal of Archaeological Sciences: Reports* (Ledger et al., 2018). This article was written by me with Dr. Mitchell as supervising author. All coauthors also provided input on the article.

The work on Sardis in section 4.3.11 has been incorporated into a manuscript submitted for publication in the *American Journal of Archaeology* (Appendix A.5). This article was written

by me and all analysis described was completed by me. Dr. Mitchell was the supervising author and all coauthors also gave their input.

Major parts of section 5.2 have been turned into a manuscript submitted for publication in the *American Journal of Archaeology* (Appendix A.5). This article was written by me and all analysis described was completed by me. Dr. Mitchell was the supervising author and all coauthors also gave their input.

Acknowledgments

I would first like to thank Dr. Mitchell for his supervision and thoughtful guidance throughout my PhD. I would also like to thank Dr. Tracy Prowse for being a supportive mentor throughout all of my academic studies and especially in my PhD.

The numerous collaborators who helped me acquire samples, shared their research, and discussed my results with me to help put them in context, without whom none of this work would have been possible. I cannot thank them enough for being so generous with their time and their openness to collaborating. Especially Erica Rowan for collaborations on a number of samples from various sites including Sardis, Aphrodisias, and Vacone.

Vagnari: Thanks to the various Vagnari excavation teams and a number of individuals and organizations who make excavation possible. In particular, the Ministero per i Beni e le Attività Culturali, the British School at Rome, Dott. Mario De Gemmis Pellicciari, Dott. Luigi LaRocca, Dottsa. Francesca Radina and Dottsa. Maria Rosaria Depalo.

Oplontis: Kristina Killgrove for collecting samples and providing osteobiographical data on the skeletal remains as well as and Nicola Terrenato (University of Michigan). The numerous organizations that make excavation possible including the Parco Archeologico Pompeii for permission to excavate the remains at Oplontis and permission to take samples of bones, teeth, and soil. John Clarke for initial discussions about material from Oplontis.

Vacone: Thanks to the various excavation teams from the Upper Sabina Tiberina Project, the superintendency for granting permission to excavate, the directors of the project Candace Rice, Gary Farney, Tyler Franconi, and Dylan Bloy, as well as Devin Ward for collecting pelvic soil samples.

Sardis: Thanks to Nicholas Cahill, Frances Gallart Marques, John Sigmier, and Erica Rowan for collection of samples and providing very detailed information on the context of the samples studied. Thanks to the various excavation teams.

Ephesus: Thanks to members of the excavating team and researchers from ÖAW. Thanks to Friederike Stock, Helmut Schwaiger, Maria Knipping, Helmut Brückner, and Sabine Ladstätter for collecting samples and providing information on context.

Viminaicum: Thanks to Nataša Šarkić and Saša Redžić for collecting samples and providing information on the context and to the excavating teams of the Viminacium Project.

Arlon: Thanks to Koen Deforce for collaborating on the samples from the Arlon latrine.

Paphos: Thanks to members of the project from the Cyprus Institute especially Kirsi Lorentz and Grigoria Ioannou for collecting samples and providing osteobiographical data on the skeletal remains.

Lauriacum: Thanks to Maria Marschler for collecting samples and providing information about the context of the cemetery.

West Smithfield: Thanks to Rebecca Redfern for collecting samples from the West Smithfield cemetery and to excavation teams.

Winterborne: Thanks to Martin Smith and the excavation teams for collecting samples for parasite analysis.

Must Farm: Thanks to the Cambridge Archaeological Unit who excavated the site. Thanks in particular to Mark Knight and Rachel Ballantyne who organized collection of samples and who were always available to discuss results of the parasite analysis and foster collaboration with other researchers working on the project. Thanks to Helen Whelton and Ian Bull who undertook the faecal lipid biomarker analysis to identify the source of the coprolites studied.

Catalhoyuk: Thanks to Scott Haddow and Chris Knüsel for providing samples to analyse and for thoughtful discussion of the results. Thanks to Evilena Anastasiou who did the initial analysis of the coprolites collected from the site.

Boncuklu: Thanks to Doug Baird for collecting samples for parasite analysis and all excavation teams.

Griffin Warrior: Thanks for Sharon Stocker, Jack Davis, and Lynne Schepartz for collecting samples for analysis and providing information about the burial and excavations.

Hatherdene Close: Thanks to the Oxford Archaeological Unit who undertook excavations of the cemetery and collected samples for parasite analysis, in particular Richard Mortimer and Natasha Dodwell.

Of course my friends and family, all of whom I will not name but I could not ask for a more loving and supportive group of people. As well as, other members of Biological Anthropology and Archaeology community at Cambridge who have made my PhD run smoothly and created a very welcoming environment to work in.

Finally, those in the Faculty of Medicine at the University of Alberta, Canada who were always so supportive of me undertaking PhD to augment my medical studies. Firstly, Sarah Forgie who encouraged me to pursue PhD in the first place and take the step to enter

the MD/PhD program. Tracey Hillier, Alan Underhill, and Peter Hamilton who made the whole program feasible. Also the late Richard Fedorak, who probably without even realizing it was one of the most encouraging people, whose enthusiasm and interest in my research kept me going when my research became very difficult, I will always value our chats about his research studying the gut microbiome of mummies.

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1 Introduction

Palaeoparasitology is defined as a branch of palaeopathology that aims to study parasites in past populations. Situated within the broader field of anthropology, palaeoparasitology uses theoretical frameworks from anthropology which aim to study ancient diseases through a lens that incorporates the relevant cultural and ecological context of the material being studied. Archaeological samples including coprolites (dried or mineralized faeces), pelvic soil samples, sediment from latrines, and mummified remains are commonly used to find evidence for parasites that infected past populations. Multiple methods can be employed in an attempt to detect parasites, these include direct visualization using light microscopy, immunological methods including enzyme linked immunosorbent assay (ELISA), and biomolecular techniques to detect DNA from the parasites themselves. While both endoparasites (parasites that live inside the body) and ectoparasites (parasites that live outside the body) can be studied in archaeological settings, this project is focused on endoparasites and more specifically gastrointestinal endoparasites.

Parasitic diseases have contributed to the disease burden of human beings throughout our evolution. Some parasites have been passed down from our primate ancestors and some have been acquired more recently as *Homo sapiens* and their hominin ancestors moved around the world and interacted with different animals and disease vectors. Today, we know that more than 1.5 billion people are infected with three of the most common intestinal parasites: roundworm, whipworm, and hookworm; and the prevalence is much higher if other parasitic diseases are included (Hotez et al., 2014). The field of palaeoparasitology has already shown that many of these parasites were present throughout human evolution and we have now begun to investigate when different species began to infect humans, how these species have been geographically distributed in the past, and what ecological, cultural, and biological factors have allowed for parasites to flourish as human pathogens. This research will contribute to these investigations by contributing new evidence and understanding of parasites in the Roman period.

Disease in the Roman period has been widely studied focusing on trauma, malaria, brucellosis, tuberculosis, and nutritional deficiencies to name a few. However, we have relatively little evidence for parasitic infection in these same populations. This project aims to remedy this situation by providing evidence for intestinal parasites in some of the understudied regions of the empire, mainly the Mediterranean and frontier provinces of the Empire (provinces located along the borders of the empire). The presence and distribution of

pathogens is influenced by cultural, social, and environmental factors that interplay with the biology of parasites and their hosts to allow for the establishment of disease. Thus, in-depth studies of pathogen presence in the past, in this case intestinal parasites, can allow for exploration of the cultural, social, and ecological determinants of disease presence. In the Roman period we are lucky to have historical texts, archaeological evidence, and human remains to aid in the interpretation of the factors shaping parasite presence and distribution. Beyond allowing for the presence of disease, many of these social and cultural factors also influence the impact these diseases may have had on people living in the Roman Empire.

Intestinal parasites could have had a major impact on the health of past populations if they were present, and there is evidence that they were present throughout much of the human past. This impact on health is due to their ability to affect their host's immune response and subsequently increase host susceptibility to other infections; and their ability to cause nutrient deficiencies by competing for nutrients and/or interrupting nutrient absorption in the host (Nematian et al., 2008; Oliveira, 2015). The effect that intestinal parasites can have on many disease processes makes them an integral piece to understanding diseases in past populations. Currently there is limited evidence for intestinal parasites in the entirety of the Roman Empire; this project aims to increase this evidence and contribute to our growing knowledge of health and disease in the Roman Empire.

1.1 Overview

The general focus of this research is to explore the presence of intestinal parasites in understudied regions of the Roman Empire. I will contribute original data to the current knowledge of parasite species that infected people living in the Roman Empire, as well as consolidate these new data with existing reports of species identified from Roman period archaeological samples, in order to create a more comprehensive view of intestinal parasitic infections in Roman populations. These data will be interpreted within their own social and ecological context, using historical and archaeological data, to understand the factors contributing to the presence of intestinal parasites in these populations. Similarly, modern biomedical and epidemiological knowledge will be used as a theoretical model to briefly discuss the impact that these parasites may have had on Roman people.

After laying out the research questions steering this research in chapter 1, the second chapter takes a look at parasitology from an evolutionary perspective to provide the necessary background information on parasitology needed to understand the organisms themselves and their evolutionary history. It then reviews published palaeoparasitological studies that provide

the existing evidence for parasites in the Roman Empire, and similarly those that have identified parasites in Europe, the Middle East, and North Africa prior to the Roman Empire, and after the fall of the Roman Empire. This is necessary to allow us to understand if the species distribution in the Roman period is different from the surrounding time periods, and in turn determine if changes that occurred in the Roman period may have had an impact on parasitic infections. For example, if a certain species of parasite found in the Neolithic and Bronze/Iron Age disappears in the Roman period, this may indicate that lifestyle changes after Roman conquest protected people from that species. Similarly, the opposite is possible, if a new species is found in Roman sites that was not previously endemic in the area this can hint towards introduction of that species perhaps via trade or migration of infected people.

There are two major methods that are well established in the field of palaeoparasitology that were used to analyse samples in this research and they are discussed in chapter 3. These are microscopy and enzyme-linked immunosorbent assay (ELISA). For microscopy, samples are suspended in solution then placed on microscope slides so that preserved parasite eggs, that would have been present in human faeces during infection, can be directly visualized. ELISA uses immunological methods to detect the antigens of smaller protozoal parasites that would similarly be found in human faeces, but are not large enough or do not preserve well enough to be visualized with microscopy.

Samples that can be used to look for evidence of ancient parasites generally include anything that contains human faecal material; this is primarily sediment samples (e.g. latrine sediments, cesspit sediments, pelvic soil, etc.), preserved tissues, and coprolites. The samples used for this research are presented in chapter 4 followed by the results; they come from Roman sites in seven different countries and more than half of these are pelvic soil. These samples have provided some of the first evidence for parasite infection in Turkey and Serbia and added to regions where very few other sites have been studied such as in Italy, Belgium, and Austria. It is necessary to compare results from Roman period sites to those of sites that pre-date and post-date the Roman period in the same regions, in order to understand if any changes occurred during the Roman period. As such, an additional set of comparative samples was also studied. Indeed some of the understudied regions of the Roman Empire have been understudied in all time periods so, where possible, samples were collected to provide additional comparative data for earlier and later time periods.

After presentation of the results of laboratory analysis of samples in chapter 4, the data is presented in discussion with collected published data from other authors in chapter 5 to understand species diversity in the Roman Empire and look for temporal changes in species

diversity. This is done on a regional basis as the samples studied in most cases form neat groups that allow for discussion of regional factors contributing to taxonomic diversity in that region and how it compares to other regions. Thus the results and discussion are presented as a series of case studies on regions that formed the focus of the research including the Eastern Mediterranean, Italy, the frontiers, and Britain. In all cases discussion of these patterns necessarily attempts to incorporate the relevant historical, archaeological, biomedical, and epidemiological evidence. As much as possible I have tried to discuss the results based on the Roman province that they are from rather than the modern day country as the Roman borders are what formed regional separations and possible cultural differences at the time. These province names are italicized throughout to try and make the distinction between modern countries and Roman provinces more clear.

In chapter 6, a broader discussion of what these parasites mean for the empire as a whole is presented as an initial effort to tie these regions together. This is first done by looking at temporal changes from the Pre-Roman, Roman, through Medieval periods in order to contextualize the parasite evidence from the Roman period and detect changes that may have occurred in the Roman period. Though there are limitations to this, mainly due to the paucity of evidence, this discussion is meant to serve as a starting point that can be further developed as research continues. This chapter then further contextualizes the results of palaeoparasitological work in the Roman period by considering what Romans understood about parasite infection, how it should be treated, and finally how it may have impacted daily life in the Roman period.

The final chapters include a brief discussion of the cautions that need to be taken with interpreting the results of this work as well as limitations of the research. Finally, I finish with suggestions for future directions in palaeoparasitology both in the Roman period and more broadly as it pertains to this research.

1.2 Research Questions

The first, overarching research question for this project is, what species of intestinal parasites were present in the Roman Empire? Further to this I will answer the questions; how were these species distributed geographically throughout the empire?; what explanations exist for any regional variations in parasite taxonomic diversity?; and were there any differences in infection between different age groups, sexes, or rural and urban populations?

The next set of research questions involves comparing the evidence gathered for parasites in the Roman Empire to what is known about parasites prior to and after the fall of

the Roman Empire, in any geographical area that was ever part of the Roman Empire. This will allow me to determine to what extent the spread of the Roman Empire impacted intestinal parasitic infections. This temporal comparison is important for determining what changes in species diversity may have been a direct result of changes to the living conditions, environment, or biology of these organisms during the Roman period. I will aim to create a broader understanding of how biological, cultural, and ecological factors during the Roman Empire may have allowed for the presence of certain species to flourish by exploring the distribution of parasite species geographically and temporally. This will be undertaken by questioning how the actions and cultural practices of a civilization can lead to changes in the infective potential of parasites and spread of these parasites to new locations.

Finally, I will explore how these parasitic infections may have impacted the daily lives of people living in the Roman Empire. Using modern biomedical knowledge as a model, I can hypothesize what impact these parasitic infections may have had on the people who had them. For example, would they have exacerbated other diseases that we know were present in the Roman period?; would they have increased the risk for other diseases?; would they have impacted an individual's ability to work?; would they have negatively impacted growth and development of children?; and so forth.

1.3 Significance and Potential Impact of Work

As will be explored further in the next section, there have been a number of studies performed that have identified parasites in the Roman Empire. However, these have been concentrated in only a few countries, and used mostly latrine samples and occupation layer sediments to look for parasites. This research will first expand on what we know about parasitic infection in the Roman period by studying samples from new countries that have, up until now, been understudied compared to other regions of the empire, and studying parasitic infection at the individual level using pelvic soil samples. Secondly, this research expands on what is known about social, cultural, and environmental contributors to health and disease in the Roman period by discussion of the presence of these parasites in combination with other archaeological, historical, and biological perspectives.

This project also has the potential to contribute to our understanding of the evolution of these parasite species alongside human beings. The field of palaeoparasitology has made continual efforts to determine when certain species first appeared in different populations around the world, and if this introduction was a result of parasites migrating with people or parasites jumping the species barrier as humans interacted with new animals and vectors as

they moved to new areas and domesticated different animals. There are still many parasite species that we have very little archaeological evidence for.

The broader goal of this work in combination with others in biological anthropology is to begin to reconstruct the presence of infectious diseases throughout human history and explore how the evolutionary trajectories of humans and pathogens have been interconnected and influenced by each other. This work, in combination with similar work in palaeoparasitology, can be used to understand the reciprocal relationships between humans and parasites. Parasites are an ideal pathogen for investigating the coevolution of humans and pathogens because of our long evolutionary history together which has resulted in many species of parasites being particularly well adapted to living in the human host.

By investigating and comparing the presence and prevalence of parasites in past populations we can understand what factors have allowed for different species to flourish as human pathogens. We can understand what environments, lifestyles, population structures, and health strategies result in higher or lower susceptibilities to infection with parasites. This in-depth understanding can help us to predict future infectivity patterns and inform modern-day decisions on where to aim preventative strategies to help the largest number of people escape the burden of parasitic disease.

2 Parasitology from an Evolutionary Perspective

Parasites have a very long evolutionary history. In certain cases fossil evidence has provided insights into very early parasites, existing well before the origins of the hominin lineage. The earliest fossil evidence for nematodes (roundworms), a type of helminth, in animals comes from the Upper Triassic period (240 million years ago [mya]) (Hugot et al., 2014), while even earlier evidence exists for cestodes (tapeworms) in a shark coprolite from the Middle Permian (275 mya) (Dentzien-Dias et al., 2013). The earliest evidence for parasites in humans comes from the Pleistocene (30,000 ya), where an egg of roundworm (*Ascaris* sp.) was found in a cave in France (Bouchet et al., 1996). The majority of evidence for parasites in the human past is much more recent, from the Neolithic period onwards. However, it is expected that the origins of many human parasites are much earlier (Poinar, 2015). Certainly, recent advances in parasite genomics is showing the significantly ancient origins of many parasites known to infect humans. In many cases, this evolutionary history extends far beyond the origins of the hominin lineage leaving many opportunities for helminths and protozoa to spread around the globe and develop necessary molecular mechanisms for surviving and thriving in diverse hosts before they were introduced to hominins (Blaxter and Koutsovoulos, 2015; Zarowiecki and Berriman, 2015).

Many parasites of humans are shared with modern primates and are thought to have been inherited from our hominin ancestors; these are sometimes called heirloom parasites (Mitchell, 2013). Parasites that have been acquired more recently as hominins moved out of Africa and were exposed to new environments and their unique ecological systems are then referred to as souvenir parasites. It is estimated that around 15% of parasites known to infect humans are human-specific while the remaining 85% are generalist pathogens that can infect many mammals (Perrin et al., 2010). Phylogenetic work and research on animal parasites has shown that some taxa were likely originally spread around the world by early animal migrations (Lotfy et al., 2008; Lawton et al., 2011). Therefore, it is not necessarily the case that humans picked up parasites in Africa and then spread them around the world with early migrations, but quite possibly that humans were first exposed in Africa and then continually exposed as they moved to other regions where the parasites had already been spread by migrations of other animals in the past, and where the climate and intermediate hosts allow the parasite to persist. Certainly this appears to be the case for certain species of schistosomiasis (e.g. *S. haematobium*) and *Fasciola*, and possibly others such as *Trichinella* and fish tapeworm (Attwood et al., 2007; Lotfy et al., 2008; Lawton et al., 2011). The exception is geographically restricted parasites (e.g. *D. pacificum*, *T. cruzi*, S. *japonicum*, *C. sinensis*) which can only exist in certain parts of the world due to the need for an intermediate

host that can only survive in certain regions of the planet. In consequence, these parasites would have only emerged as human diseases when humans migrated to their endemic region. For further discussion of the evolution of parasites and their presence throughout human history see Ledger and Mitchell (2019). We will go into specific details on the characteristics of these different parasites in the following subsection.

Genetic evidence, particularly in the form molecular phylogenetic studies, highlights the ancient origins of many parasites and their ubiquity throughout much of human evolution. However, archaeological evidence from palaeoparasitological studies has pointed to various human actions that have changed the diseases we experience or the prevalence of these diseases through time. This is where we are focused when exploring parasite infection in the Roman period.

2.1 Relevant Intestinal Parasites Causing Disease in Humans

Today, there are around 300 species of parasites known to infect man (Cox, 2002). For practical reasons, we cannot look for all of these species in past populations and indeed some of them are quite rare. However, the methods used to look for parasites in past populations allow us to look for a large number of these, and in particular we have been successful in finding intestinal parasites in many time periods and locations around the world as the eggs of these parasites are quite robust and preserve well in archaeological sediments. Mummified remains can be used to find evidence for even more species that do not always preserve well in other material.

Parasites can be broken up into two broad categories: endoparasites and ectoparasites. Endoparasites are parasites that live inside the body. They are mainly helminths (worms), for example roundworm and hookworm, and protozoa such as *Giardia* and *Entamoeba*. On the other hand, ectoparasites generally live on the skin or in superficial layers of the skin, for example ticks and lice. While there is evidence for ectoparasites in the archaeological record, they are typically found in different materials than endoparasites. For example, lice may be found on preserved hair of a mummy or on combs. Ectoparasites are not typically considered to cause infection themselves but rather infestation. However, they can transmit other pathogens (i.e. bacteria, viruses, protozoa) which can cause serious infections. Due to the fact that ectoparasites do not cause infection themselves, this project is focused on endoparasites, which can cause true infection on their own.

Endoparasites can be broken up into two distinct groups: Protozoa and Metazoa (helminths) (Levinson, 2016). Protozoa are generally much smaller and are unicellular,

whereas helminths are multicellular worms. There are multiple subtypes of Protozoa including amoebas, sporozoas, flagellates, and ciliates. Helminths are further subdivided into two Phyla: Nemathelminthes (nematodes or roundworms), and Platyhelminthes (flatworms) which can be broken down into two clades; trematodes (flukes) and cestodes (tapeworms) (Figure 1). In the following sections, we will explore the relevant human parasites in each of these groups. Most parasites that will be discussed have both a common name as well as their scientific name. In the remaining sections common names will be used as much as possible for ease of understanding; however scientific names are often required to indicate the taxonomic level to which parasites were identified.

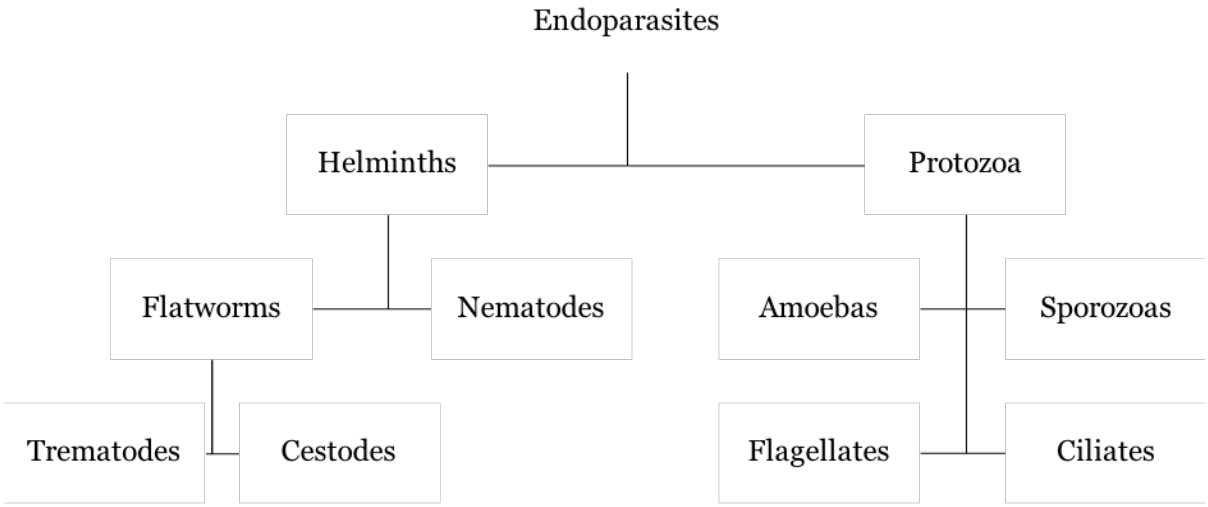

Figure 1: Classification of endoparasites causing infection in humans. After Levinson (2016).

2.1.1 Nematodes

Intestinal nematodes are the most common species of parasites infecting humans worldwide (Hotez et al., 2014). Nematodes or roundworms are nonsegmented worms that have both male and female forms and possess an intestinal tract. As with most helminths the adult form is found in the definitive host, in this case humans, where reproduction occurs. During the developmental process the nematodes also require a period of time to mature outside of the definitive host. The exact life cycle varies between different species but generally consists of five stages of development including multiple larval stages that then develop into mature adults (Weller, 2018).

Table 1 describes various characteristics of the most common human nematodes, including the endemic region, the route of infection, the major sites of infection in the body, and the most common clinical symptoms. Figure 2 includes images of common intestinal nematodes that infect humans. It is important to note that many nematode infections are

asymptomatic, or only begin with minor symptoms as the parasite migrates through the body to its final destination, followed by a longer asymptomatic period. More serious and in some cases life-threatening symptoms develop if the individual is continuously reinfected and is host to a large number of helminths (Garcia, 2016). The major method of detection in palaeoparasitology is also listed and corresponds to the site of infection and principles of reproduction. Microscopy is useful for parasites that release eggs from the host in the faeces or urine, which can then be identified in coprolites and sediments containing faecal material. Nematodes like guinea worm and *Trichinella* spp. can only be diagnosed if the adult worms or cysts which contain larvae are found in preserved tissues, as these nematodes do not release eggs into the faeces.

Table 1: Characteristics of relevant human nematodes (Weller and Nutman, 2018; Garcia, 2016)

Nematode	Endemic Region	Route of Infection	Site of Infection	Clinical Signs and Symptoms	Method of Detection
Ascaris sp. (roundworm)	Worldwide	Ingestion of eggs	Intestinal	Pneumonitis, fever, cough, intestinal obstruction, abdominal pain, nausea/vomiting,	Microscopy of faeces
Trichuris trichiura (whipworm)	Worldwide	Ingestion of eggs	Intestinal	Diarrhoea, anaemia, abdominal pain	Microscopy of faeces
Ancylostoma duodenale/ Necator americanus (hookworm)	Southern Europe, Africa, Asia, Americas (in areas with warm moist climates)	Larvae penetrate skin	Intestinal	Pruritus at entry site, pneumonitis, fatigue, nausea/vomiting, abdominal pain, diarrhoea, weakness, pallor, iron deficiency anaemia	Microscopy of faeces
Enterobius vermicularis (pinworm)	Worldwide	Ingestion of eggs	Intestinal	Anal pruritus	Microscopy of faeces
Strongyloides stercoralis	Tropical	Larvae penetrate skin; autoinfection	Intestinal	Larva migrans, Loeffler's syndrome, diarrhoea, anorexia, abdominal pain, hyperinfection syndrome	Microscopy of faeces
Capillaria spp.	Worldwide (depends on species)	Ingestion of larvae from contaminated foods	Intestinal	Diarrhoea, weight loss, abdominal pain	Microscopy of tissue or faeces

Trichostrong-ylus spp.	Worldwide	Ingestion of larvae on plants	Intestinal	Abdominal pain, diarrhoea, anorexia, nausea, dizziness, fatigue	Microscopy of faeces
Dioctophyma renale (giant kidney worm)	Worldwide	Ingestion of raw freshwater fish or frogs	Kidney	Kidney failure, abdominal pain	Microscopy of faeces/urine
Dracunculus medinensis (guinea worm)	Africa (Asia in the past)	Ingestion of infected copepod (water fleas)	Subcutan-eous tissues	Rash, pruritus, nausea, vomiting, diarrhoea, blisters	Microscopy of preserved tissue
Trichinella spp.	Worldwide	Ingestion of raw or undercooked meat from infected mammals	Striated muscle	Diarrhoea, nausea, abdominal pain, fever, muscle pain, weakness, specific symptoms depending on area infected	Microscopy of preserved tissue

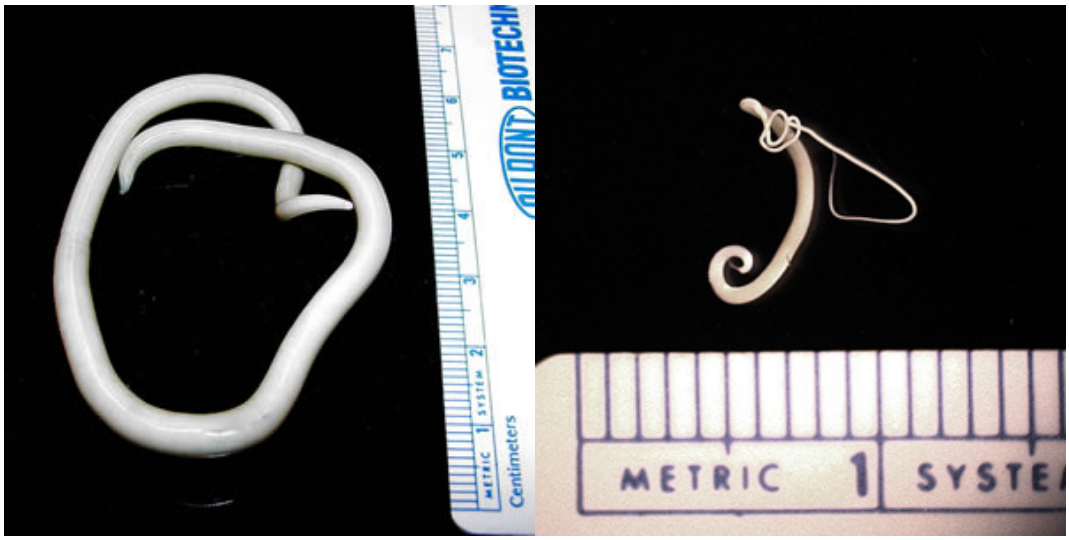

Figure 2: Images of common intestinal nematodes. Left image is an adult roundworm (*Ascaris* sp.). Image from CDC (2019), https://www.cdc.gov/dpdx/ascariasis/index.html.
Right image is an adult whipworm (*Trichuris trichiura*). Image from CDC (2017), https://www.cdc.gov/dpdx/trichuriasis/index.html

2.1.2 Cestodes

Cestodes, commonly known as tapeworms, are segmented worms made up of a head, which can attach to the intestinal mucosa, and a varying number of proglottids. These proglottids possess both male and female sexual organs, allowing them to become pockets of eggs that can be released from the host (Weller, 2018). The life cycle of cestodes involves an intermediate host besides humans. When these animals ingest eggs released from the definitive host the eggs hatch and the larvae migrate through tissues to form a cysticercus in

varying tissues or organs. When the definitive host then ingests any tissue containing cysticerci the parasite completes its life cycle and develops into a mature adult in the intestinal tract.

Table 2 lists some of the most common cestodes that infect human beings and that can be found in archaeological samples. Figure 3 includes images of common intestinal cestodes that infect humans. Most tapeworms that infect humans are acquired through ingestion of undercooked meat containing larvae. One exception is *Echinococcus granulosus*, also known as hydatid disease. Infection with *E. granulosus* in humans is caused by ingestion of eggs rather than infected animal meat. This is because humans are an intermediate host for *E. granulosus* not the definitive host, which is usually dogs (Garcia, 2016). When eggs passed in the faeces of dogs are swallowed, cysts containing the larvae develop in the tissues of humans. Similarly, humans can become accidental intermediate hosts to *Taenia solium* (pork tapeworm) if eggs from the faeces of oneself or other people are ingested, usually as a result of poor hand hygiene. Space occupying cysts, that contain parasite larvae, can then develop in almost any area of the body but often occur in the brain, a disease known as neurocysticercosis.

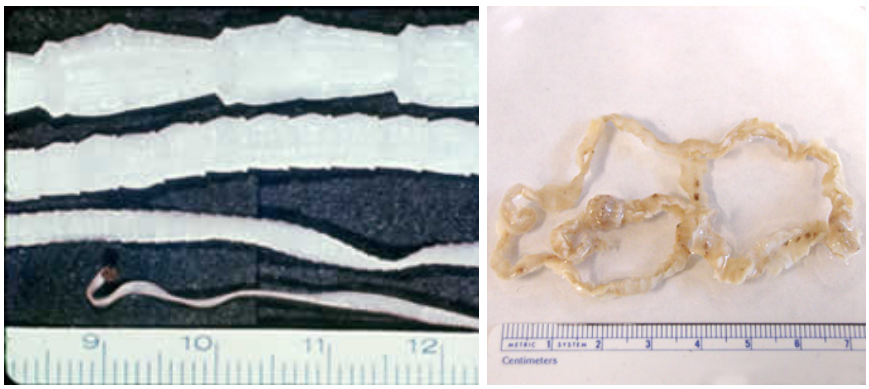

Figure 3: Images of common intestinal cestodes. Left is an image of an adult beef tapeworm (*Taenia saginata*). Image is from CDC (2017), https://www.cdc.gov/dpdx/taeniasis/index.html. Right is an image of an adult fish tapeworm (*Diphyllobothrium latum*). Image is from CDC (2019), https://www.cdc.gov/dpdx/diphyllobothriasis/index.html

Table 2: Characteristics of relevant human cestodes (White and Weller, 2018; Garcia, 2016)

Cestode	Endemic Region	Route of Infection	Site of Infection	Clinical Signs and Symptoms	Method of Detection
Taenia saginata (beef tapeworm)	Worldwide	Raw or undercooked beef	Intestinal	Diarrhoea, intestinal obstruction, weight loss, appendicitis	Microscopy of faeces
Taenia solium (pork tapeworm)	Worldwide	Raw or undercooked pork	Intestinal and tissue	Indigestion, diarrhoea, constipation, cysticercosis	Microscopy of faeces
Echinococcus granulosus complex (hydatid disease)	Worldwide	Ingestion of eggs	Tissue (usually lungs and liver)	Symptoms from enlarging cysts dependent on tissue location, allergic reactions	Microscopy of preserved tissue/cyst
Hymenolepis nana (dwarf tapeworm)	Worldwide	Ingestion of eggs; autoinfection	Intestinal	Headache, anorexia, abdominal pain, diarrhoea	Microscopy of faeces
Diphyllobothrium spp. (fish tapeworm)	Worldwide	Raw or undercooked fish	Intestinal	Intestinal obstruction, diarrhoea, abdominal pain, megaloblastic anaemia	Microscopy of faeces
Spirometra spp.	Worldwide	Ingestion of contaminated water, raw or undercooked fish, reptiles, amphibians	Tissue	Quite varied depending on location in body	Microscopy of faeces

2.1.3 Trematodes

Trematodes, commonly known as flukes, are the second major group of flatworms. Most trematodes are hemaphroditic, meaning they contain both male and female sex organs. Once eggs are released from the definite host their life cycle involves different species of snails as an intermediate host (Vennervald, 2018). The larvae released from the snails can either penetrate the skin of the definitive host directly or are ingested from the snail or another intermediate host. Once in the definitive host (humans or other mammals) the trematodes can live in blood, biliary ducts, intestines, or the lungs.

Table 3: Characteristics of relevant human trematodes (Vennervald, 2018; Garcia, 2016)

Trematode	Endemic Region	Route of Infection	Site of Infection	Clinical Signs and Symptoms	Method of Detection
Schistosoma spp.	South America, Caribbean, Africa, the Middle East, Southeast Asia	Cercariae penetrate skin	Blood vessels around bladder and intestines	Cercarial dermatitis, acute schistosomiasis (Katayama syndrome), tissue fibrosis from trapped eggs, colonic obstruction	Microscopy of faeces/urine
Fasciola spp. (*Fasciola* liver fluke)	Worldwide	Ingestion of metacercariae on aquatic plants	Bile ducts	Abdominal pain, fever, rash, hepatosplenomegaly, inflammation of liver/gallbladder/ pancreas, biliary obstruction	Microscopy of faeces
Echinostoma spp.	Worldwide	Ingestion of molluscs, snails, freshwater fish	Intestines	Localized inflammation, ulceration, diarrhoea, abdominal pain	Microscopy of faeces
Dicrocoelium dendriticum (Lancet liver fluke)	Europe, Turkey, northern Africa, northern Asia, North America, South America	Ingestion of infected ants	Bile ducts	Chronic constipation, flatulence, jaundice, hepatomegaly, diarrhoea, vomiting	Microscopy of faeces
Opisthorchis felineus	Southeast Asia, Eastern Europe	Ingestion of raw or undercooked fish	Bile ducts	Abdominal pain, diarrhoea, constipation, cholangitis, cholangiocarcinoma	Microscopy of faeces

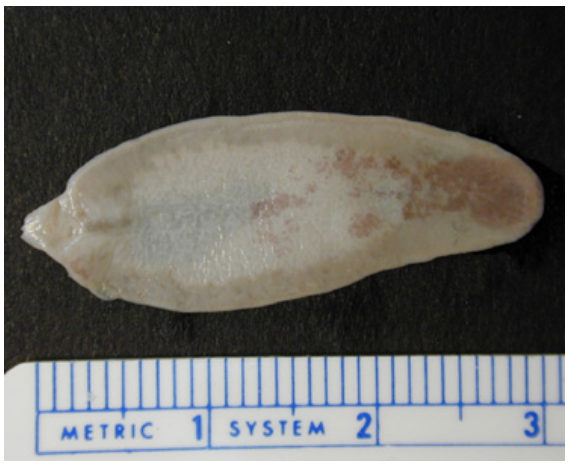

Figure 4: Image of an adult *Fasciola* liver fluke worm (*Fasciola hepatica*). Image is from CDC (2019), https://www.cdc.gov/dpdx/fascioliasis/index.html.

Table 3 lists characteristics of some of the most common trematodes that infect humans. Though many of these species are not primarily found in the intestines, eggs can migrate through the body tissues and are still released in the faeces (as in the case of *Schistosoma mansoni*), or they are released into the urine (as in the case of *Schistosoma haematobium*) and can be found during microscopic analysis of sediment samples contaminated with human excrement. For the organisms that live in the bile ducts, eggs are carried through the intestines with bile and can be found in the faeces of infected individuals. There are also cases where eggs are primarily found in urine though these can often be detected in the same archaeological samples that contain faeces.

2.1.4 Protozoa

Table 4: Characteristics of relevant human protozoa (Garcia, 2016)

Protozoan	Endemic Region	Route of Infection	Site of Infection	Clinical Signs and Symptoms	Method of Detection
Entamoeba histolytica (amoebic dysentery)	Worldwide	Ingestion of faecal contamina-ted water or food	Intestinal	Bloody diarrhoea, colitis, appendicitis, amoebic granulomas, toxic megacolon, liver abscess, peritonitis	ELISA
Giardia duodenalis	Worldwide	Ingestion of faecal contamina-ted water or food	Intestinal	Diarrhoea, abdominal pain, nausea, vomiting	ELISA
Cryptosporidium spp.	Worldwide	Ingestion of faecal contamina-ted water or food	Intestinal	Watery diarrhoea, abdominal pain, nausea, vomiting, fever, weight loss	ELISA
Leishmania spp.	Africa, Asia, Middle East, South America	Sand fly bite	Cutaneous or visceral	Cutaneous: nodular and ulcerative skin lesions; Visceral: fever, malaise, weight loss, diarrhoea, lymphadenopathy, hepatosplenomegaly, darkening of skin	aDNA
Plasmodium spp. (malaria)	Worldwide in the past; today in the tropics and subtropics mainly	*Anopheles* mosquito bite	Liver and red blood cells	cyclical fever, anaemia, hepatomegaly	aDNA

Protozoa are the other major group of endoparasites alongside helminths. They are unicellular organisms that do not lay eggs but reproduce via asexual reproduction and are often preserved in their inactive cyst form in the environment. There are a number of protozoal species that are some of the most common causes for diarrhoea today. These include: the only pathogenic amoeba *Entamoeba histolytica*, the flagellate *Giardia duodenalis* (synonym *Giardia lamblia*), and the sporozoan *Cryptosporidium* spp. (Garcia, 2016). These three organisms live in the intestines and release cysts, trophozoites, or oocysts in the faeces which can survive in moist environmental conditions and then infect new individuals if they are ingested.

The other common protozoa found in the past that cause infections in humans are *Leishmania* spp., which cause leishmaniasis, and *Plasmodium* spp. which cause malaria. The life cycle of these parasites is much more complicated and so far the most successful methods for detecting their presence in past populations relies on ancient DNA (aDNA) analysis of preserved tissue or bone.

2.2 Palaeoparasitology

Palaeoparasitology has been said to have started with early work done by Marc Armund Ruffer in 1910 when he identified eggs of the blood fluke, *Schistosoma haematobium,* in an Egyptian mummy dated between 1250–1000 BCE. In fact, many of the first studies in palaeoparasitology used mummified remains to look for ancient parasites. Szidat (1944) looked for parasite eggs in the intestinal remains of two bog bodies from Prussia. In the body of a young woman known as Drobnitz girl, dated to 600 BCE, eggs from whipworm (*Trichuris* sp.) and roundworm (*Ascaris* sp.) were found. In the body of a man known as Karwinden man, dated to 500 CE, eggs from whipworm, roundworm, and fish tapeworm (*Diphyllobothrium latum*) were found. Taylor (1955) was the first to highlight the value in studying archaeological sediments from excavated latrines. Eggs of human roundworm (*Ascaris* sp.), human whipworm (*Trichuris trichiura*), and the Lancet liver fluke (*Dicrocoelium dendriticum*) were found in a medieval pit from Winchester, Britain. Callen and Cameron (1960) published a landmark paper in the field describing methods for rehydrating and analysing coprolites for parasite eggs. Around the same time, Witenberg (1961) studied coprolites, for evidence of ancient parasites. Witenberg analysed coprolites from Nahal-Mishmar valley in Israel and found human whipworm, and possible dysentery-causing protozoa (*Entamoeba histolytica* and *Giardia duodenalis*). More recently, palaeoparasitologists have begun to advocate for the use of pelvic soil to look for ancient parasites (Reinhard et al., 1992; Bouchet et al., 2001a; Fugassa et al., 2006). Throughout this time, palaeoparasitology has established itself as its own field and larger questions exploring the evolution and spread of parasites around the world have become the focus of palaeoparasitologists. There are now various labs dedicated to palaeoparasitology around the world that have contributed to a steadily increasing body of work on parasite presence in past populations and what this can tell us about our ancestors.

Palaeoparasitological studies have been used to answer many questions about our human ancestors and the evolution of human parasites. These range from tracking parasite introduction to new regions to trace early human migrations and detecting the origins of human parasites, to the wider spread of parasites around the globe, and more specifically

identifying parasites present at various points in human evolutionary history. One well known example of the usefulness of investigations in palaeoparasitology is the evidence for hookworm in the New World and what it means for the peopling of the Americas. Evidence of hookworm has been found in South America from 7,000 years before present (BP) (Araújo et al., 1988). Many explanations were proposed for how hookworm reached the Americas, however as an obligate human parasite they all centred around how people could have arrived in the Americas with hookworm infection. The life cycle of hookworm requires the eggs to hatch in the soil and the larvae to develop in a warm and moist environment, this ruled out hookworm entering the Americas with early human migrations via the Bering land bridge because hookworm would not have been able to survive the cool arctic temperatures. Rather, Araújo and colleagues (2008) proposed that hookworm entered the Americas with later coastal or transpacific migrations. In another similar example, Yeh and colleagues (2016) found Chinese liver fluke (*Clonorchis sinensis*) on hygiene sticks from a relay station along the Silk Road. Due to the location of this station in a region of China where this parasite cannot survive this provided support for the movement of the parasite with an individual travelling along the Silk Road from areas of eastern or southern China where the parasite is endemic.

Understanding the origins of parasites has also been a focus of palaeoparasitologists. Mitchell (2013) reviewed the evidence gained from various palaeoparasitological studies to conclude when certain species were seen first in different parts of the world and then infer if these parasites are heirloom parasites (passed down from our human ancestors in Africa) or souvenir parasites (acquired later as we moved out of Africa). While palaeoparasitology has shown its value in understanding past human populations there are still many questions we have yet to answer about the origins of some common human parasites as well as the presence of parasitic disease in past populations. Particularly for this research we still do not understand what parasitic infections were present in many parts of the Roman Empire.

2.3 Palaeoparasitological Review Relevant to the Roman Empire

The Roman imperial period has been a heavily studied period in human history and has long held a great interest for many people. With expansion of the Roman Empire, most of Europe, the Near East, and even some of Africa went through a period of changing social norms and mixing of cultures, as existing cultures melded with the newly introduced Roman cultural norms and social practices. As the extent of the Roman Empire increased there were corresponding increases in the movement of goods and individuals throughout Europe and the surrounding areas (Ligt and Tacoma, 2016). This trade and migration had inevitable impacts

on the spread of disease and likely parasite infections as well. As archaeological excavations were taken up at Roman period sites, the analysis of parasites at these sites was achieved both as the primary goal of research and as secondary outcomes of other analyses, such as archaeobotanical or palynological analysis.

In this section I will review the relevant studies that have looked for evidence for endoparasites in Europe, the Middle East, and North Africa. At the height of the Roman Empire, the borders of the empire extended into the Middle East and into North Africa (Figure 5), thus these regions must be included in our review. First, I will look at what evidence exists from the Neolithic period up until the Roman period. I will then review the evidence that we already have for parasites at Roman period sites. Finally, I will review the evidence we have for parasites in Europe, the Middle East, and North Africa after the Roman period. I have organized the parasite discussed in each major time period by transmission category including, faecal oral helminths, zoonotic cestodes (tapeworms), zoonotic trematodes (flukes), protozoa, and other parasites. The main zoonotic cestodes found in the time periods discussed are beef/pork tapeworm (*Taenia* spp.) and fish tapeworm (*Diphyllobothrium* spp.), thus these are included in the zoonotic cestodes sections. The main zoonotic trematodes found in the time periods discussed are liver flukes including the lancet liver fluke (*Dicrocoelium dendriticum*) and *Fasciola* liver fluke, thus these are the trematodes included in the zoonotic trematodes sections. To maintain consistency between time periods, other parasites found infrequently are included in the other parasites sections.

It is important to note that in some cases published reports have no photos of the parasite eggs found so their results cannot be confirmed. Furthermore, many studies were unable to determine if the parasites identified came from humans or animals. This evidence has been included in the review if it could reasonably come from a human context but its uncertainty is noted where necessary.

Figure 5: Map of Roman provinces in 117 CE. Provincial boundaries from the Digital Atlas of Roman and Medieval Civilizations, Harvard University.

As with any literature review there is certain evidence that has been excluded from this review or needs to be interpreted with caution. As much as possible I have reported the parasites found as identified by the authors of the studies. One consideration to be taken is the debate around the distinction between *Ascaris lumbricoides* and *Ascaris suum*, which affects how these species are reported. It has been common to assume that roundworm eggs from human contexts must be from the human species (*A. lumbricoides*) rather than pig roundworm (*A. suum*), however recent analysis of the genomes of these organisms have pointed out considerable similarities between the two and questioned their classification as distinct species (Nejsum et al., 2012; Betson et al., 2014). Furthermore, both *A. lumbricoides* and *A. suum* can infect humans so roundworm could be considered zoonotic in some cases. Despite this, it is classified as a faecal-oral parasite as this is the most common route of transmission between humans. Some authors report eggs in human contexts as *A. lumbricoides* due to the context of the material studied and some eggs are not identified to the species level. As much as possible I have chosen to keep the authors' level of identification however, in my own work roundworm eggs are not identified to the species level but recorded as *Ascaris* sp. Another consideration to bear in mind is that many older publications do not include photos making it difficult to confirm their results, as such I have chosen to say that authors have identified these eggs. Some commonly cited evidence has been excluded from the review

where I felt that recent advances in parasitology have made the evidence unreliable as in the case of microscopic identification of *Entamoeba histolytica* cysts (Witenberg, 1961), as these cannot be distinguished from cysts of the non-pathogenic organism *Entamoeba dispar.* Finally, where I have counted the number of taxa identified the number reflects the minimum number of unique organisms identified either to the genus or species level. So, if multiple species from the same genus were reported these are counted as separate taxa but an additional genus level identification is not counted as an additional taxon; whereas in cases where parasites are identified to the genus level these are counted as one taxon.

To the best of my abilities I have included all evidence for parasites that have been found in regions that came under Roman rule. However, it is possible that there is some evidence missing, especially from excavation reports where mention of parasites was made in environmental studies and where these reports are not in English. In many cases *Ascaris* sp. and *Trichuris* sp. are identified in palynological analyses as very recognizable parasite eggs, therefore it is possible that including a large number of these reports may skew the data to emphasize these parasites if uncommon parasites were missed or unidentified in these cases.

2.3.1 Parasites in the Palaeolithic Period

Preserved parasite eggs from the Palaeolithic have been found at one site in France. Human roundworm eggs (*Ascaris lumbricoides*) were identified from samples taken in the cave of Grand-Grotte at Arcy-sur-Cure (28,000–22,600 BCE) (Bouchet et al., 1996). The eggs were determined to be human roundworm because there was no evidence of swine or boar in the area, the definitive hosts of *Ascaris suum*. There was evidence for the presence of humans and bears in the cave, however *Ascaris* spp. are not known to infect bears, leaving humans as the most likely hosts for the eggs found in the cave.

2.3.2 Parasites in the Mesolithic Period

Similar to the Palaeolithic period there is little evidence for parasites in the Mesolithic. The only evidence for parasites that comes from a region that later fell under control of the Roman Empire is from Wales (Dark, 2004). Samples from intertidal peat deposits were studied and found to contain eggs of *Trichuris* sp. These samples were radiocarbon dated to 5840–5620 BCE which corresponds to the late Mesolithic in this region. It is important to note that these eggs came from environmental samples so could be *T. suis* from pigs or *T. trichiura* from humans.

2.3.3 Parasites in the Neolithic Period

Though there is some variation in exactly what dates encompass the Neolithic, for the purposes of this work I have included sites in Neolithic Europe that date between 6,500–2,500 BCE, if the time period was not otherwise specified by the authors (Fowler et al., 2015). This covers the period from the earliest known Neolithic site in Greece to the spread of a Neolithic lifestyle into northern Europe. When we start to look at countries in the Middle East the Neolithic period is defined a bit differently due to the difference in progression of material culture in that area, therefore evidence included for the Neolithic period from sites in the Middle East are dated between 10,000–3,300 BCE.

Table 5: Parasites found at Neolithic period sites.

Country	Site	Date	Parasites	Sample Type	Citation
Cyprus	Shillouroka-mbos	8300–7000 BCE	*Ascaris lumbricoides,* *Diphyllobothrium* sp., *Fasciola* sp., *Taenia* sp., *Trichuris trichiura*	Pelvic soil	Harter-Lailheugue et al., 2005
	Khirokitia	7000–6000 BCE	*Ascaris lumbricoides,* *Fasciola* sp., *Taenia* sp., *Trichuris trichiura*	Pelvic soil	Harter-Lailheugue et al., 2005
France	Clairvaux	3600 BCE	*Ancylostomatidae*, *Diphyllobothrium* sp., *Fasciola* sp., *Trichuris* sp.	Sediment	Dommelier-Espejo, 2001
	Chalain	3200–2980 BCE	*Capillaria* sp., *Dicrocoelium* sp., *Diphyllobothrium* sp., *Fasciola hepatica*, *Taenia* sp., *Trichuris trichiura*	Coprolites/ occupation sediment	Bouchet et al., 1995a; Bouchet, 1997; Dommelier et al., 1998; Dommelier-Espejo, 2001

Germany	Leipzig-Zwenkau	5259–5258 BCE	*Dicrocoelium* sp.	Pit	Le Bailly and Bouchet, 2010
	Hornstaad-Hornle	3917–3905 BCE	*Dicrocoelium* sp., *Diphyllobothrium* sp., *Fasciola* sp., *Trichuris* sp.	Occupation sediment	Le Bailly, 2005
	Sipplingen	3711-3306 BCE	*Dicrocoelium* sp., *Diphyllobothrium* sp., *Fasciola* sp., *Taenia* sp., *Trichuris* sp.	Occupation sediment	Le Bailly, 2005
	Wallhausen-Ziegelhütte	3700–2900 BCE	*Capillaria* sp., *Diphyllobothrium* sp., *Fasciola* sp., *Trichuris* sp.	Occupation sediment	Le Bailly, 2005
	Torwiesen II	3283–3281 BCE	*Capillaria* sp., *Diphyllobothrium* sp., *Fasciola* sp., *Taenia* sp., *Trichuris* sp.	Occupation sediment	Le Bailly, 2005
	Seekirch-Stockwiesen	3000–2900 BCE	*Capillaria* sp., *Dicrocoelium* sp., *Diphyllobothrium* sp., *Fasciola* sp., *Trichuris* sp.	Occupation sediment	Le Bailly, 2005
	Karsdorf	3000–2000 BCE	*Fasciola hepatica*	Pelvic soil	Dittmar and Teegen, 2003
	Alleshausen-Taschenwiese	2900–2600 BCE	*Dicrocoelium* sp.	Occupation sediment	Le Bailly and Bouchet, 2010
Greece	Kouphovouno	5000–2000 BCE	*Entamoeba histolytica*	Pelvic soil	Le Bailly and Bouchet, 2006
	Kephala	4000–3000 BCE	*Trichuris trichiura*	Pelvic soil	Anastasiou et al., 2018
Italy	Otzi mummy	3300–3200 BCE	*Trichuris trichiura*	Mummy	Aspöck et al., 1996
Netherlands	Swifterbant	3400–3230 BCE	*Capillaria* sp., *Fasciola* sp., *Opisthorcis* sp., Oxyuridae, *Trichuris* sp.	Coprolites	Roever-Bonnet et al., 1979
	Amsterdam	3400–3230 BCE	*Trichuris* sp.	?	Jansen and Boersma, 1972

Switzerland	Concise	3700 BCE	*Dicrocoelium* sp., *Entamoeba histolytica*	Occupation sediment	Le Bailly and Bouchet, 2010; Le Bailly and Bouchet, 2015
	Arbon	3384–3370 BCE	*Ascaris* sp., *Capillaria* sp., *Dicrocoelium* sp., *Dioctophyma renale*, *Diphyllobothrium* sp., *Entamoeba histolytica*, *Fasciola* sp., *Opisthorchis* sp., *Taenia* sp., *Trichuris* sp.	Coprolites	Dommelier-Espejo, 2001; Le Bailly et al., 2003a; Gonçalves et al., 2004
	Parkhaus-Opéra, Zürich	3176–3153 BCE	*Ascaris* sp., *Capillaria* sp., *Dicrocoelium* sp., *Diphyllobothrium* sp., *Echinostoma* sp., *Fasciola gigantica*, *Taenia*/*Echinococcus* sp., *Trichuris trichiura*	Occupation sediment	Flammer et al., 2018; Maicher et al., 2019
Spain	La Draga	5320–4980 BCE	*Ascaris* sp., *Capillaria* sp., *Dicrocoelium dendriticum*, *Diphyllobothrium* sp., *Enterobius vermicularis*, *Maracanthorhynchus* sp., *Taenia saginata*, *Trichuris trichiura*	Occupation sediment	Maicher et al., 2017
Syria	Tell Zeidan	4550–4050 BCE	*Schistosoma* sp.	Pelvic soil	Anastasiou et al., 2014

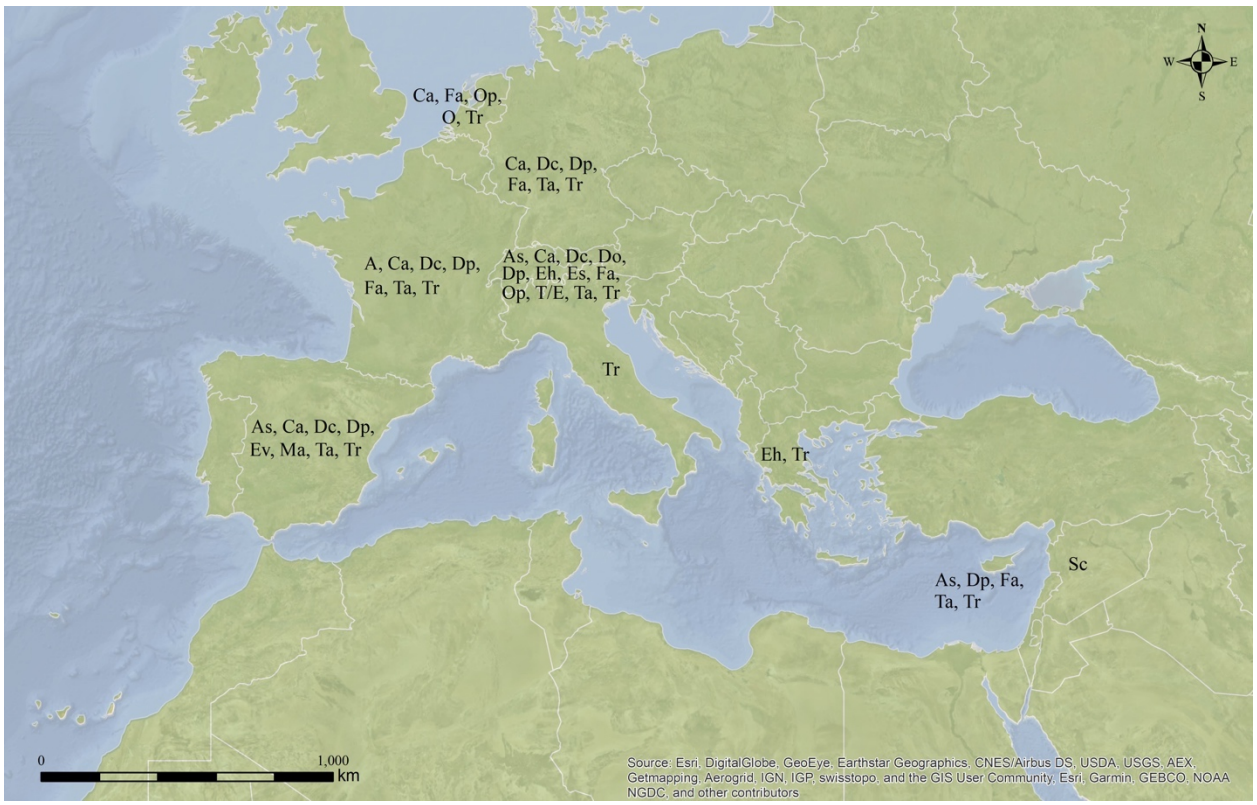

Figure 6: Parasites found at Neolithic period sites, mapped onto modern-day countries. A=*Ancylostomatidae*, As=*Ascaris*, Ca=*Capillaria*, Dc=*Dicrocoelium*, Do=*Dioctophyma*, Dp=*Diphyllobothrium*, Eh=*Entamoeba histolytica*, Es=*Echinostoma*, Ev=*Enterobius vermicularis*, Fa=*Fasciola*, Ma=*Maracanthorhynchus*, O=*Oxyuridae*, Op=*Opisthorchis*, Sc=*Schistosoma*, T/E=*Taenia*/*Echinococcus*, Ta=*Taenia*, Tr=*Trichuris*.

In the Neolithic period, the intestinal parasites that have been identified from archaeological contexts are *Ancylostomatidae*, *Capillaria* sp., *Dicrocoelium* sp. liver fluke, *Echinostoma* sp., *Entamoeba histolytica, Fasciola* liver flukes, fish tapeworm, giant kidney worm, *Maracanthorhynchus* sp., *Opisthorchis* sp., Oxyuridae, pinworm, roundworm, *Schistosoma* sp., *Taenia* tapeworms, and whipworm. These results have come from studies performed at 22 different sites. The sites that have been studied, sample details, and parasites identified can be found in Table 5. The taxa found in each country are summarized in Figure 6. While most of these cases are presumed to be a result of human infection, a large number of parasite remains from these sites come from occupation sediments thus we cannot be sure if these parasites came from animals or humans.

2.3.3.1 Faecal-Oral Helminths

Roundworm (*Ascaris* sp.) has been found at 5 of the 22 Neolithic period sites studied. Whipworm (*Trichuris* sp.) has been found at 16 Neolithic period sites. Interestingly in the

Neolithic period we see whipworm found in the absence of roundworm more often than in later time periods.

Roundworm (*Ascaris lumbricoides*) and whipworm (*Trichuris trichiura*) eggs have been found at two sites in Cyprus, Shillourokambos and Khirokitia. These eggs were found alongside other zoonotic taxa in pelvic soil from burials at the two sites dating between 8300–6000 BCE (Harter-Lailheugue et al., 2005).

Roundworm (*Ascaris* sp.) and whipworm (*Trichuris* sp.) were also found at the site of Arbon in Switzerland alongside other zoonotic taxa. These eggs were identified in coprolites dating between 3384–3370 BCE (Dommelier-Espejo, 2001; Le Bailly et al., 2003a; Gonçalves et al., 2004). Also in Switzerland, sitewide analysis of occupation sediment (Maicher et al., 2019) and aDNA analysis (Flammer et al., 2018) has provided evidence for roundworm and whipworm as well as six other taxa at the site of Parkhaus-Opéra in Zürich.

In Spain, roundworm (*Ascaris* sp.) and whipworm (*Trichuris trichiura*) were found in occupation layer sediments from the site of La Draga. This was a lakeside settlement dating between 5320–4980 BCE (Maicher et al., 2017).

Whipworm was found in the absence of roundworm at two sites in France, Clairvaux and Chalain. At Clairvaux, *Trichuris* sp. eggs were identified in occupation layer sediments (Dommelier-Espejo, 2001) and at Chalain *Trichuris trichiura* eggs were identified in coprolites and sediment (Bouchet et al., 1995a).

In Germany, a series of sediment samples from a number of sites throughout the Neolithic period were studied by Le Bailly (2005) and reported in his PhD thesis. The sites are dated between 3917–2900 BCE. Roundworm was not found at any of these sites though whipworm (*Trichuris* sp.) eggs were found at all of these sites. Whipworm eggs were not found at the three sites in Germany (Leipzig-Zwenkau, Karsdorf, and Alleshausen-Taschenwiese) studied by other authors (Dittmar and Teegen, 2003; Le Bailly and Bouchet, 2010).

Whipworm (*Trichuris trichiura*) has been found in pelvic soil from the site of Kephala in Neolithic Greece (Anastasiou et al., 2018) and in a mummy from Neolithic Italy (Aspöck et al., 1996), both in the absence of any other taxa. The mummy from Italy was that of Otzi, who was discovered naturally mummified in the Alps on the border between Austria and Italy. He is thought to have lived between 3300–3200 BCE. Faecal samples taken from his intestines contained the whipworm eggs (Aspöck et al., 1996).

In the Netherlands, whipworm (*Trichuris* sp.) eggs were found in coprolites dating between 3400–3230 BCE from the site of Swifterbant alongside other zoonotic taxa (Roever-Bonnet et al., 1979) as well as in an unknown sample type from Amsterdam dated to 3400–3230 BCE (Jansen and Boersma, 1972).

Another helminth often found alongside roundworm and whipworm in modern populations is hookworm, as these are all soil-transmitted helminths. *Ancylostomatidae* eggs have been identified from the site of Clairvaux in France dated to 3600 BCE (Dommelier-Espejo, 2001). However, these potential *Ancylostomatidae* eggs were only identified to the Family level and are not necessarily human hookworm. Further they should be interpreted with caution as hookworm eggs are very fragile and do not often preserve in archaeological contexts.

2.3.3.2 Zoonotic Cestodes

The two types of tapeworms that have been found in Neolithic Europe and the Middle East are fish tapeworm (*Diphyllobothrium* sp.) and beef/pork tapeworms (*Taenia* spp.). Fish tapeworm was been found at 11 sites studied and beef/pork tapeworm at 8 sites.

Fish tapeworm eggs were found in pelvic soil samples from the site of Shillourokambos in Cyprus dating between 8300–7000 BCE (Harter-Lailheugue et al., 2005). They were found at both Clairvaux and Chalain in France at later time periods between 3600–2980 BCE (Dommelier-Espejo, 2001; Bouchet et al., 1995a). They have also been found in occupation layer sediments from the five sites in Germany studied by Le Bailly (2005) for his thesis. In Switzerland, fish tapeworm eggs were found in coprolites from the site of Arbon (3384–3370 BCE) (Dommelier-Espejo, 2001) and in occupation sediment from Parkhaus-Opéra, Zürich (3176–3153 BCE) (Maicher et al., 2019). They were also found in occupation layer sediment from the lakeside settlement of La Draga in Spain, dating between 5320–4980 BCE (Maicher et al., 2017).

Beef and pork tapeworms (*Taenia* spp.) were found in pelvic soil from Shillourokambos (8300–7000 BCE) and Khirokitia (7000–6000 BCE) in Cyprus (Harter-Lailheugue et al., 2005). They were found in coprolites from Chalain in France (Dommelier et al., 1998). They were found in two of five sites studied in Germany by Le Bailly (2005). These were Sipplingen (3711-3306 BCE) and Torwiesen II (3283–3281 BCE).

Taenia sp. eggs were found alongside fish tapeworm (*Diphyllobothrium* sp.) at Arbon and Parkhaus-Opéra, Zürich in Switzerland. They were also found alongside fish tapeworm at La Draga in Spain, and in this case sequencing of helminth DNA retrieved using multiplex

PCR from the samples confirmed that these eggs belonged the beef tapeworm (*Taenia saginata*) (Maicher et al., 2017).

2.3.3.3 Zoonotic Trematodes

Trematodes (flukes) including the lancet liver fluke (*Dicrocoelium dendriticum*) and *Fasciola* liver fluke (*Fasciola* sp.) have been found in many Neolithic period sites in Europe and the Middle East. *Dicrocoelium* sp. eggs have been found at 10 Neolithic period sites in Europe and the Middle East and *Fasciola* sp. eggs have been found at 13 sites. *Dicrocoelium* sp. eggs were found at sites in France, Germany, Switzerland, and Spain. *Fasciola* sp. eggs were found at sites in Cyprus, France, Germany, Netherlands, and Switzerland. While most eggs are not identified to the species level due to similar morphology with other species in the same genus, it is expected that most *Dicrocoelium* eggs in Europe are from the lancet liver fluke (*Dicrocoelium dendriticum*) because that is the only species found in Europe today (Le Bailly and Bouchet, 2010).

Dicrocoelium sp. eggs and *Fasciola hepatica* eggs were found together at the site of Chalain, France dating between 3200–2980 BCE (Bouchet et al., 1995a; Bouchet, 1997; Dommelier et al., 1998; Dommelier-Espejo, 2001). *Dicrocoelium* sp. eggs have been found at five Neolithic sites in Germany including Leipzig-Zwenkau, Hornstaad-Hornle, Sipplingen, Seekirch-Stockwiesen, and Alleshausen-Taschenwiese (Le Bailly, 2005; Le Bailly and Bouchet, 2010). These were all found in occupation layer sediments except for the eggs from Leipzig-Zwenkau, which were found in sediment from a pit. These sites date from 5259 to 2000 BCE. In Switzerland, *Dicrocoelium* eggs were found at all three sites that have been studied including Concise, Arbon, and Parkhaus-Opéra, Zürich (Dommelier-Espejo, 2001; Le Bailly and Bouchet, 2010; Maicher et al., 2019). These eggs come from occupation layer sediment and coprolites from the 4th millennium BCE. Finally in Spain, lancet live fluke (*Dicrocoelium dendriticum*) eggs were found at the lakeside settlement of La Draga (5320–4980 BCE) (Maicher et al., 2017).

Fasciola liver fluke was found in pelvic soil samples from both sites that have been studied from Neolithic Cyprus; Shillourokambos and Khirokitia dating from 8300 to 6000 BCE (Harter-Lailheugue et al., 2005). *Fasciola* liver fluke was found in occupation layer sediment from Clairvaux in France (3600 BCE) and *Fasciola hepatica* was found in coprolites and occupation layer sediment from the site of Chalain in France (3200–2980 BCE) (Dommelier-Espejo, 2001). *Fasciola* sp. eggs have been found at six sites in Neolithic Germany including Hornstaad-Hornle, Sipplingen, Wallhausen-Ziegelhütte, Torwiesen II,

Seekirch-Stockwiesen, and Karsdorf (Le Bailly, 2005). All of these eggs were found in occupation layer sediment except for those from Karsdorf which were found in pelvic soil from burials dated between 3000–2000 BCE (Dittmar and Teegen, 2003). In the Netherlands, *Fasciola* liver fluke eggs have been found in coprolites from the site of Swifterbant (Roever-Bonnet et al., 1979). In Switzerland, they were found in coprolites from Arbon (Dommelier-Espejo, 2001). Also in Switzerland, *Fasciola gigantica* eggs were found in occupation layer sediment from Parkhaus-Opéra, Zürich (Maicher et al., 2019). However, it is important to note that the samples studied from Parkhaus-Opéra, Zürich were sediments from occupation layers, and none of the parasites found are human-specific and could have been a result of animal infection at the site. The presence of *Paramphistomum* sp. in some of these samples suggests the presence of animal faecal material in some areas.

2.3.3.4 Protozoa

The only protozoan parasite so far found in Neolithic Europe and the Middle East is *Entamoeba histolytica*. *Entamoeba* was detected from both the site of Arbon (3384–3370 BCE) and the site of Concise (3700 BCE) in Switzerland using ELISA (Le Bailly and Bouchet, 2015). At Arbon it was detected in coprolites and at Concise it was detected in occupation layer sediments. In Greece, *Entamoeba histolytica* was found in pelvic soil from burials at Kouphovouno dating between 5000-2000 BCE (Le Bailly and Bouchet, 2006).

2.3.3.5 Other Parasites

Other helminths that have been found less commonly in the Neolithic period compared to those already mentioned are *Capillaria* sp., *Opisthorchis* sp., *Maracanthorhynchus* sp., *Dioctophyma renale*, *Echinostoma* sp., *Schistosoma* sp., and Oxuyuridae.

Capillaria sp. worm has been found at 8 sites in the Neolithic period. These sites are located in France, Germany, Netherlands, Switzerland, and Spain. First, *Capillaria* sp. eggs were found in coprolites and sediment from Chalain in France (Bouchet et al., 1995a). They were found in occupation layer sediment from three sites in Germany (Le Bailly, 2005). These three sites were Wallhausen-Ziegelhütte, Torwiesen II, and Seekirch-Stockwiesen. They were found in the coprolites studied from Swifterbant in the Netherlands (Roever-Bonnet et al., 1979). They were found at both Arbon and Parkhaus-Opéra, Zürich in Switzerland (Dommelier-Espejo, 2001; Maicher et al., 2019). Finally, they were found in occupation layer sediments from La Draga in Spain (Maicher et al., 2017). Most evidence for *Capillaria* sp. in this time period comes from occupation layer sediment however, there is

also some evidence from coprolites. It is unclear if these are all cases of human infection as *Capillaria* sp. can infect many mammals. Furthermore, its presence in human coprolites may not represent true infection but may be a result of human ingestion of liver from infected animals where the eggs are found.

Opisthorchis sp. eggs have been found in coprolites from Arbon, Switzerland and Swifterbant, Netherlands. Similarly, *Maracanthorhynchus* sp, a common parasite of animals has been found at La Draga in Spain.

In Switzerland, the site of Arbon was a rich source for ancient parasites; ten taxa were found at this site including the only case of giant kidney worm (*Dioctophyma renale*) in archaeological samples from Europe (Le Bailly et al., 2003a). This egg was found in a coprolite, however the eggs are typically released in the urine which could indicate some mixing of urine in the faeces or false parasitism with ingestion of an egg that did not hatch in the intestines of an individual.

Also in Switzerland, *Echinostoma* sp. was found at Parkhaus-Opéra, Zürich (Maicher et al., 2019). This is a rare parasite in the archaeological record which has also been found in Mesolithic Ireland (Perri et al., 2018).

Pelvic soil from a burial in Syria gives us the only evidence for schistosomiasis in the Neolithic period (Anastasiou et al., 2014). The species of schistosome was suggested to be either *S. intercalatum* or *S. haematobium*. The geographical distribution of these two species is restricted to warmer climates and is not often found in Europe today but is endemic in Africa and parts of the Middle East, the lack of sites studied in these endemic regions in the Neolithic period is likely the reason we have little evidence for it. Another species, *Schistosoma bovis* has been detected in the southern Mediterranean where it has also been shown to hybridize with *S. haematobium* in humans. These hybrid infections have recently been recorded in Corsica, France (Oleaga et al., 2019).

Pinworm, is another very common parasite today that is transmitted by ingestion of eggs. Eggs are often present on hands after itching the anus and can be transmitted to food or other objects to later be ingested. Eggs can also be found and transmitted through objects such as bedding or clothing of an infected individual. At the site of Swifterbant in Switzerland, *Oxyuridae* eggs were found in coprolites (Roever-Bonnet et al., 1979). These eggs could only be identified to the Family level and could be from human pinworm. Interestingly, the only other evidence for pinworm in Europe is from the Neolithic site of La Draga in Spain (Maicher et al., 2017)) until the Medieval period; although we have cases of it from Sudan starting in the Bronze Age. The eggs of pinworm have thin eggshells that are thought to be

less resistant to degradation and thus not found very commonly in Europe and the Middle East.

Overall, in Neolithic Europe there is a large diversity of parasite taxa found. With most sites having more than one species present and some having as many as ten different taxa at one site. Comparatively, there has been much less work done in Neolithic Middle East and North Africa compared to Europe.

2.3.4 Parasites in the Bronze Age

Relatively little is known about parasites in Bronze Age and Iron Age Europe. For the purposes of this work, I have included samples dating between 2,500–600 BCE in the evidence for Bronze Age Europe, where the time period was not already specified. As with the Neolithic period, the dates encompassing the Bronze Age in the Middle East and Northern Africa vary slightly from that of Europe, as such for this region I have included evidence from samples that date between 3300–1200 BCE (Liverani, 2007). Six taxa have been identified from five different European countries including Austria, Britain, the Czech Republic, France, and Greece; these are *Dicrocoelium* liver fluke, *Entamoeba histolytica,* fish tapeworm, hookworm, roundworm, and whipworm. Most of the work undertaken on Bronze Age parasites from countries outside Europe that at one point fell under Roman rule is in Egypt and Sudan. In these countries a minimum of twelve taxa have been found including *Dicrocoelium* liver fluke, *Fasciola* liver fluke, filarial worms, guinea worm, *Leishmania* sp., pinworm, roundworm, *Schistosoma haematobium, Schistosoma mansoni*, *Taenia* sp., *Trichinella spiralis*, and whipworm.

Table 6: Parasites found at Bronze Age sites.

Country	Site	Date	Parasites	Sample Type	Citation
Austria	Hallstatt salt mines	800–350 BCE	*Ascaris lumbricoides, Trichuris trichiura*	Coprolites	Aspöck et al., 1973a; Aspöck et al., 1973b
Britain	Brean Down	1940–650 BCE	*Ascaris lumbricoides*, *Trichuris trichiura*	Coprolites	Jones, 1990
Czech Republic	Hulín	1600–1500 BCE	*Ancylostoma duodenale*, *Ascaris lumbricoides*, *Trichuris trichiura*	Pelvic soil	Šebela et al., 1990
Egypt	Mummy BM32753	3200 BCE	*Schistosoma haematobium*	Mummy	Deelder et al., 1990
	Mummies from Thebes	2050–1650 BCE	*Leishmania donovani*	Mummy	Zink et al., 2006
	Mummy from Valley of Nobles	1450 BCE	*Dracunculus medinensis*	Mummy	Horne and Redford, 1995
	Mummy	1250–1000 BCE	*Schistosoma haematobium*	Mummy	Ruffer, 1910
	Mummy Natsef Amun	1200 BCE	Lymphatic filariasis	Mummy	Tapp and Wildsmith, 1992
	Mummy Nakht-ROM I	1200 BCE	*Schistosoma* sp., *Taenia* sp., *Trichinella spiralis*	Mummy	Reyman et al., 1977; Boni et al., 1977
	Mummies from Thebes	1198–1150 BCE	*Schistosoma haematobium*	Mummy	Deelder et al., 1990
France	Grésine, Bourget Lake	3400–3230 BCE	*Dicrocoelium* sp., *Diphyllobothrium* sp., *Entamoeba histolytica*	Occupation sediment	Gonçalves et al., 2004; Le Bailly and Bouchet, 2010; Le Bailly and Bouchet, 2013
Greece	Ayia Irini	1600–1100 BCE	*Ascaris* sp.	Pelvic soil	Anastasiou et al., 2018

Sudan	Sai Island	2700 BCE	*Ascaris* sp., *Enterobius vermicularis*, *Schistosoma* sp.	Pelvic soil	Harter, 2003
	Kerma	2400–1750 BCE	*Ascaris lumbricoides*, *Dicrocoelium* sp., *Fasciola* sp., *Schistosoma haematobium*, *Schistosoma mansoni*, *Taenia* sp., *Trichuris trichiura*	Pelvic soil	Harter, 2003

Figure 7: Parasites found at Bronze Age sites, mapped on modern-day countries. An=*Ancylostoma*, As=*Ascaris*, Dc=*Dicrocoelium*, Dp=*Diphyllobothrium*, Dr=*Dracunculus medinensis*, Eh=*Entamoeba histolytica*, Ev=*Enterobius vermicularis*, Fa=*Fasciola*, Le=*Leishmania*, Lf=Lymphatic filariasis, Sc=*Schistosoma*, Ta=*Taenia*, Tr=*Trichuris*, Ts=*Trichinella spiralis.*

2.3.4.1 Faecal-Oral Helminths

Roundworm and whipworm have also been found at sites in the Bronze Age. Roundworm (*Ascaris* sp.) has been found at 6 sites and whipworm (*Trichuris* sp.) has been found at 4 sites. Coprolites from the Bronze Age studied from the Hallstatt salt mines give us evidence for roundworm (*Ascaris lumbricoides*) and whipworm (*Trichuris trichiura*) in Austria (Aspöck et

al., 1973a, 1973b). Similarly, only one site from Bronze Age Britain has been studied for intestinal parasites; this is the site of Brean Down, Somerset. Sediment samples and coprolites were studied and revealed eggs of roundworm (*Ascaris lumbricoides*) and whipworm (*Trichuris trichiura*) (Jones, 1990). The eggs were presumed to be from human infections because the size of the whipworm eggs fit within the range for modern human whipworm. This is the earliest evidence that currently exists for parasites in Britain.

In the Czech Republic, evidence for intestinal parasites in the Bronze Age comes from pelvic soil from burials at the site of Hulín, in which hookworm, roundworm (*Ascaris lumbricoides*) and whipworm (*Trichuris trichiura*) were reported in pelvic soil samples (Šebela et al., 1990). The report of hookworm (*Ancylostoma duodenale*) is unusual as this is a fragile egg not often found in archaeological sites in Europe. This is in part because of its thin eggshell but also due to the fact that the eggs hatch in the soil and larvae penetrate the skin of humans directly, meaning that numerous unhatched eggs would not be expected if the parasite is completing its life cycle.

From the site of Ayia Irini in Greece roundworm (*Ascaris* sp.) eggs were found in pelvic soil from one of two individuals analysed that were buried during the Bronze Age (Anastasiou et al., 2018).

Finally, pelvic soil from Bronze Age sites in Sudan have contributed evidence for roundworm at Sai Island (2700 BCE) and Kerma (2400–1750 BCE) (Harter, 2003). Whipworm was also found in pelvic soil from Kerma.

2.3.4.2 Zoonotic Cestodes

Cestodes (tapeworms) including fish tapeworm (*Diphyllobothrium* sp.) and beef/pork tapeworm (*Taenia* sp.) have been found at Bronze Age sites in Europe, the Middle East, and North Africa.

In Egypt, a mummy known as Nakht-ROM I dating to 1200 BCE, was shown to be infected with *Taenia* tapeworm (Reyman et al., 1977). The only other evidence for *Taenia* sp. tapeworms in the Bronze Age comes from pelvic soil from the site of Kerma (2400–1750 BCE) in the Sudan (Harter, 2003).

In France, our understanding of parasites in the Bronze Age comes from the site of Grésine near Bourget Lake (3400–3230 BCE). The site was a lakeside settlement in France; compared to the other sites studied in Bronze Age Europe it appears that its proximity to

freshwater may have affected the taxa of parasite found there as this is the only site where fish tapeworm (*Diphyllobothrium* sp.) was found (Le Bailly and Bouchet, 2013).

2.3.4.3 Zoonotic Trematodes

Trematodes (flukes) have been found at a few Bronze Age sites. The most common is *Dicrocoelium* sp. which has been found at two sites. *Fasciola* liver fluke has been found at one site. This is far fewer than in the Neolithic period though there is more evidence for helminths in the Neolithic period in general.

Dicrocoelium liver fluke was also found at the lakeside settlement of Grésine, France. The eggs were found in occupation layer sediment dated to 3400–3230 BCE (Le Bailly and Bouchet, 2010). Pelvic soil from Bronze Age Kerma in the Sudan contributed evidence for *Dicrocoelium* liver fluke and *Fasciola* liver fluke (Harter, 2003).

2.3.4.4 Protozoa

The only evidence for gastrointestinal protozoa in the Bronze Age comes from the site of Grésine in France where *Entamoeba histolytica* was detected in occupation layer sediment (Gonçalves et al., 2004).

2.3.4.5 Other Parasites

Other parasites that have been found in Bronze Age sites from Europe, the Middle East, and North Africa include *Dracunculus medinensis, Leishmania donovani,* lymphatic filariasis, pinworm (*Enterobius vermicularis*), schistosomiasis (*Schistosoma* sp.), and *Trichinella spiralis*. Many of these more rare species in archaeological contexts have been found in mummified remains from Egypt where worms and larvae that live in tissue have the chance to preserve along with eggs.

Pelvic soil from Sai Island in Sudan has contributed the only evidence for pinworm in the Bronze Age. Also in pelvic soil from Sail Island eggs from *Schistosoma* sp. were found. In pelvic soil from Kerma, Sudan eggs from *Schistosoma haematobium* and *Schistosoma mansoni* have been identified (Harter, 2003).

In Egypt, most of the evidence we have for parasitism has come from the autopsy of high status mummies. Guinea worm (*Dracunculus medinensis*) was found in a mummy from the Valley of the Nobles dated to 1450 BCE (Horne and Redford, 1995). Lymphatic filariasis, caused by one of three species, *Wuchereria bancrofti*, *Brugia malayi*, or *Brugia timori* was

found in the mummy Natsef Amun dated to 1200 BCE (Tapp and Wildsmith, 1992). DNA analysis has confirmed one of the earliest cases of leishmaniasis from Thebes in ancient Egypt. DNA of *Leishmania donovani* was amplified from bone tissues from four mummies dated between 2050–1650 BCE (Zink et al, 2006). Other mummies from Thebes have been shown to have schistosomiasis. The first dates to 1198–1150 BCE (Deelder et al., 1990) and the second the mummy Nakht-ROM I dates to 1200 BCE (Reyman et al., 1977). Nakht-ROM I was also shown to be infected with *Taenia* tapeworm and *Trichinella spiralis* (Boni et al., 1977). Finally, Ruffer (1910) reported finding eggs of *Schistosoma haematobium* in an Egyptian mummy dated between 1250–1000 BCE, in what is commonly noted as the first paleoparasitological study undertaken. Overall, there are many unique species identified in Egypt during the Bronze Age including, guinea worm, leishmaniasis, and lymphatic filariasis. However, this is in part a result of differential preservation of parasites in different sample types; in Egypt most evidence for parasite infection has come from the autopsy of mummies whereas in other areas sediment samples are the dominant sample type studied. Mummified remains offer the possibility to identify the adult worms of various parasites as well as eggs or cysts preserved in soft tissue.

2.3.5 Parasites in the Iron Age

For the Iron Age material, I have included samples from Europe that date between 600 BCE–100 CE and 1200–200 BCE for the Middle East and North Africa, where the time period is not otherwise specified by the authors. As can be seen there is very little evidence for parasites in the Iron Age compared to any other time period. Paleoparasitological studies on Iron Age sites have revealed eggs from 11 unique taxa in four different countries (Table 7 and Figure 8). These include *Dicrocoelium* liver fluke, *Echinococcus* sp., *Fasciola* liver fluke, fish tapeworm, pinworm, roundworm, *Schistosoma haematobium*, *Schistosoma mansoni*, *Strongyloides* sp., *Taenia* tapeworm, and whipworm.

Table 7: Parasites found at Iron Age sites.

Country	Site	Date	Parasites	Sample Type	Citation
Austria	Hallein salt mine	500–200 BCE	*Ascaris* sp., *Dicrocoelium* sp., *Fasciola hepatica;* *Taenia* sp., *Trichuris trichiura*	Coprolites	Aspöck et al., 1973a; Aspöck et al., 1973b; Aspöck et al., 1999; Le Bailly and Bouchet, 2010
Egypt	Mummy Asru from Thebes	750–525 BCE	*Echinococcus* sp., *Strongyloides* sp.	Mummy	Tapp, 1984
	Deir el-Medineh	400–300 BCE	*Ascaris lumbricoides*, *Diphyllobothrium* sp., *Fasciola hepatica,* *Trichuris trichiura*	Pelvic soil	Harter, 2003
	Saqqara	400–300 BCE	*Diphyllobothrium* sp., *Fasciola* sp., *Trichuris trichiura*	Pelvic soil	Harter, 2003
	Mummy	200–1 B.C.E	*Taenia* sp.	Mummy	Bruschi et al., 2006
	Mummy Pum II	170 BCE	*Ascaris lumbricoides*	Mummy	Cockburn et al., 1975
Israel	City of David, Jerusalem	700–500 BCE	*Taenia* sp., *Trichuris trichiura*	Latrine	Cahill et al., 1991
Sudan	Sai Island	700–300 BCE	*Ascaris lumbricoides*, *Diphyllobothrium* sp., *Enterobius vermicularis*, *Fasciola* sp., *Schistosoma haematobium*, *Schistosoma mansoni*, *Taenia* sp., *Trichuris trichiura*	Pelvic soil	Harter, 2003

Figure 8: Parasites found at Iron Age sites, mapped onto modern-day countries. As=*Ascaris*, Dc=*Dicrocoelium*, Dp=*Diphyllobothrium*, Ec=*Echinococcus*, Ev=*Enterobius vermicularis*, Fa=*Fasciola*, Sc=*Schistosoma*, St=*Strongyloides*, Ta=*Taenia*, Tr=*Trichuris*.

2.3.5.1 Faecal-Oral Helminths

Roundworm (*Ascaris* sp.) has been found four sites in the Iron Age and whipworm (*Trichuris* sp.) has been found at five sites. The only Iron Age site studied from Europe is the Hallein salt mines in Austria (500–200 BCE). In coprolites from the site, roundworm (*Ascaris* sp.) and whipworm (*Trichuris trichiura*) eggs were found (Aspöck et al., 1973a; Aspöck et al., 1973b; Aspöck et al., 1999). Roundworm and whipworm eggs were also found in pelvic soil from Deir el-Medineh in Egypt (Harter, 2003) and in pelvic soil from Iron Age Sai Island in the Sudan (Harter, 2003).

Roundworm (*Ascaris lumbricoides*) was the only helminth found in a mummy known as Pum II from Egypt (Cockburn et al., 1975). This mummy is dated to 170 BCE.

Whipworm (*Trichuris trichiura*) was found in the absence of roundworm in pelvic soil samples from Saqqara in Egypt (Harter, 2003), as well as in a latrine from Jerusalem, Israel (Cahill et al., 1991).

2.3.5.2 Zoonotic Cestodes

Cestodes (tapeworms) have been found a six of the eight sites studied in the Iron Age. Beef/pork tapeworms (*Taenia* sp.) have been found at four sites and fish tapeworm (*Diphyllobothrium* sp.) at three sites.

Taenia sp. eggs were found in coprolites from the Hallein salt mines in Austria (500–200 BCE) (Aspöck et al., 1999). In Iron Age Egypt, pelvic soil taken from burials at Deir el-Medineh and Saqqara revealed eggs of fish tapeworm (*Diphyllobothrium* sp.) (Harter, 2003). Also, an Egyptian mummy dated to 200–1 BCE was found to have a cyst containing the larvae of pork tapeworm and was confirmed using immunochemical staining (Bruschi et al., 2006).

In the latrine from Jerusalem, eggs from beef/pork tapeworm (*Taenia* sp.) were found alongside whipworm (Cahill et al., 1991). This latrine was dated to 700–500 BCE.

Pelvic soil from burials on Sai Island in the Sudan contained similar species to Bronze Age burials in Kerma and Sai Island. However, in the Iron Age *Dicrocoelium* liver fluke was not found and fish tapeworm (*Diphyllobothrium* sp.) was found in addition to the other taxa seen one of which is beef/pork tapeworm (*Taenia* sp.) (Harter, 2003).

2.3.5.3 Zoonotic Trematodes

Flukes have been found in coprolites, mummies, and pelvic soil from the Iron Age. *Dicrocoelium* sp. eggs and *Fasciola hepatica* eggs were found in coprolites from the Hallein salt mines in Austria (Aspöck et al., 1999; Le Bailly and Bouchet, 2010). Pelvic soil from Deir el-Medineh and Saqqara in Egypt contained eggs from *Fasciola* liver fluke (Harter, 2003). *Fasciola* sp. eggs were also found in pelvic soil from Sai Island in the Sudan (Harter, 2003).

2.3.5.4 Other Parasites

Other parasites that have been found in the Iron Age include *Echinococcus* sp. which causes hydatid disease, pinworm (*Enterobius vermicularis*), schistosomiasis (*Schistosoma haematobium* and *Schistosoma mansoni*), and *Strongyloides* sp.

In the mummy named Asru from Thebes (750–525 BCE) a hydatid cyst indicating *Echinococcus* sp. infection was found along with evidence for infection with *Strongyloides* sp. (Tapp, 1984). In the pelvic soil from Iron Age Sai Island (700–300 BCE) eggs of pinworm

and two species of *Schistosoma* (*Schistosoma haematobium* and *Schistosoma mansoni*) were identified (Harter, 2003).

2.3.6 Parasites in the Roman Empire

Currently 13 different taxa of parasites have been identified from Roman period sites and these come from 12 countries including Austria, Belgium, Britain, Egypt, France, Germany, Greece, Israel, Italy, the Netherlands, Switzerland, and Turkey (Table 8). These taxa include *Capillaria* sp., *Dicrocoelium* liver fluke, *Echinococcus* sp., *Entamoeba histolytica*, *Fasciola* liver fluke, fish tapeworm, *Giardia duodenalis*, *Macracanthorhynchus* sp., pinworm, roundworm, *Taenia* tapeworms, *Toxocara* sp., and whipworm. Though *Oxyuris equi* (horse pinworm) has been found at some of these sites and in a number of Roman samples (Dufour, 2015), I have not included it in further discussions as it is not known to infect humans. While this seems like a fair number of studies there are still major gaps in our knowledge of the entirety of the Empire. Of the 53 Roman sites where parasites have been reported, 30 (57%) are in Britain and France. Egypt is the only country that has been studied from Roman North Africa. Though the heart of the Roman Empire lies in Italy, only three sites in Italy have been studied for intestinal parasites (the number increases to six if malaria is included). Similarly, there are no data from Spain, Portugal, Hungary, Croatia and most countries in the Eastern Mediterranean (Figure 9). In order to draw conclusions about which parasites were present in the Roman Empire, and how changes occurring during this time impacted parasite diversity, we need to have a more even spread of data from across the Empire.

Whipworm (*Trichuris* sp.) is the most common parasite found in Roman period sites, being found at 34 sites. Roundworm (*Ascaris* sp.) is also very common, being found at 31 sites. The next most common parasite found is *Fasciola* liver fluke followed by the lancet liver fluke (*Dicrocoelium* sp.), beef/pork tapeworm (*Taenia* sp.), fish tapeworm (*Diphyllobothrium* sp.), and *Capillaria* sp. worm. Other less common helminths that have been found include hydatid disease (*Echinococcus granulosus*), pinworm (*Enterobius vermicularis*), and *Toxocara* sp. Intestinal protozoa including *Entamoeba histolytica* and *Giardia duodenalis* have also been found, as well as other protozoa including malaria (*Plasmodium falciparum*) and toxoplasmosis (*Toxoplasma gondii*).

Table 8: Parasites found at sites in the Roman Empire.

Country	Roman Province	Site	Date	Parasites	Sample Type	Citation
Austria	*Pannonia Superior*	Carnuntum	101–300 CE	*Ascaris lumbricoides, Taenia* sp., *Trichuris trichiura*	Sewer and latrine	Aspöck et al., 2011; Petznek, 2018
Belgium	*Belgica*	Arlon	1–300 CE	*Ascaris* sp., *Trichuris* sp.	Vats or pits	Defgnée et al., 2008; Gourevitch et al., 2011
	Belgica	Mageroy	Roman	*Entamoeba histolytica*	Latrine	Le Bailly and Bouchet, 2015
Britain	*Britannia*	Leicester	1–200 CE	*Ascaris* sp., *Fasciola* sp., *Trichuris* sp.	Cesspit	Boyer, 1999
	Britannia	London, Hibernia Wharf	1–200 CE	*Ascaris lumbricoides*, *Diphyllobothrium latum*, *Taenia* sp., *Trichuris trichiura*	Well	Rouffignac , 1985
	Britannia	Orton Longueville	1–200 CE	*Echinococcus granulosus*	Cyst from skeleton	Wells and Dallas, 1976
	Britannia	Carlisle	1–300 CE	*Ascaris lumbricoides*, *Fasciola* sp., *Trichuris trichiura*	Occupation sediment	Jones and Hutchison, 1991
	Britannia	Ambleside	1–400 CE	*Ascaris* sp., *Trichuris trichiura*	Pit	Jones, 1985
	Britannia	Church Street Sewer, York	1–500 CE	*Ascaris* sp., *Trichuris* sp.	Sewer	Wilson and Rackham, 1976
	Britannia	London, 15–35 Copthall Ave.	101–400 CE	*Dicrocoelium dendriticum*, *Trichuris trichiura*	Occupation sediment	De Moulins, 1990
	Britannia	Bearsden	142–158 CE	*Ascaris* sp., *Trichuris* sp.	Sewer	Knights et al., 1983

	Britannia	Lincoln, Waterside NW	301–400 CE	*Trichuris* sp.	Occupation sediment	Carrott et al., 1995
	Britannia	Owslebury, Winchester	Roman	*Ascaris* sp., *Dicrocoelium* sp., *Trichuris*/*Capillaria* sp.	Pit	Pike, 1968
	Britannia	Poundbury, Dorset	Roman	*Ascaris lumbricoides*, *Trichuris trichiura*	Coffin sediment	Jones, 1987
Egypt	*Aegyptus*	Dakhleh Oasis	395 BCE–30 CE	*Enterobius vermicularis*	Mummy	Horne, 2002
	Aegyptus	El-Deir	301–500 CE	*Taenia* sp.	Mummy	Le Bailly et al., 2010
France	*Lugdunensis*	Bobigny hospital	201 BCE–100 CE	*Ascaris lumbricoides*, *Trichuris trichiura*	Pelvic soil	Rousset et al., 1996
	Narbonensis	Marseille	14 BCE–27 CE	*Diphyllobothrium* sp., *Fasciola* sp., *Trichuris* sp.	Occupation sediment	Harter-Lailheugue, 2006
	Narbonensis	Lattes	1–200 CE	*Entamoeba histolytica*	Cesspit	Le Bailly and Bouchet, 2006
	Lugdunensis	Beauvais	1–300 CE	*Ascaris* sp., *Capillaria sp.,* *Dicrocoelium* sp., *Macracanthorhynchus* sp., *Taenia*/*Echinococcus* sp., *Trichuris* sp.	Latrine	Dufour, 2015
	Germania Superior	Horbourg-Wihr	1–300 CE	*Ascaris* sp., *Capillaria* sp., *Dicrocoelium* sp., *Diphyllobothrium sp.,* *Fasciola* sp., *Macracanthorhynchus* sp., *Taenia*/*Echinococcus* sp., *Trichuris* sp.	Pits, latrines, ditches	Dufour, 2015
	Aquitania	Bordeaux	40–51 CE	*Ascaris* sp., *Taenia* sp., *Trichuris* sp.	Sewer or latrine	Sireix, 2008

Belgica	Reims, Rue Venise	101–200 CE	*Ascaris* sp., *Fasciola* sp., *Trichuris* sp.	Pits, wells, occupation sediment	Bouchet et al., 2001b; Le Bailly et al., 2003b
Aquitania	Jaunay-Clan	201–300 CE	*Trichuris trichiura*	Coffin sediment	Dufour et al., 2016
Narbonensis	La Gramiere, Castillon du Gard	201–300 CE	*Entamoeba histolytica*	Cesspit	Gonçalves et al., 2004
Lugdunensis	Amiens	201–401 CE	*Capillaria hepatica*, *Echinococcus granulosus*	Cyst from skeleton	Mowlavi et al., 2014
Germania Superior	Andilly-en-Bassigny	Roman	*Ascaris* sp., *Fasciola* sp., *Taenia*/*Echinococcus* sp.	?	Dufour, 2015
Lugdunensis	Autun	Roman	*Ascaris* sp., *Capillaria* sp., *Macracanthorhynchus* sp., *Taenia*/*Echinococcus* sp., *Trichuris* sp.	?	Dufour, 2015
Lugdunensis	Evreux	Roman	*Ascaris* sp., *Trichuris* sp.	Pelvic soil	Dufour, 2015
Lugdunensis	Lisses	Roman	*Entamoeba histolytica*	Pit	Le Bailly and Bouchet, 2015
Belgica	Metz	Roman	*Ascaris* sp., *Capillaria* sp., *Diphyllobothrium* sp., *Fasciola* sp. *Toxocara* sp., *Trichuris* sp.	?	Dufour, 2015
Aquitania	Mikelauen-Zilo	Roman	*Dicrocoelium* sp., *Diphyllobothrium* sp.	Occupation sediment	Le Bailly and Bouchet, 2010; Le Bailly and Bouchet, 2013
Belgica	Reims, Rue Carnot	Roman	*Dicrocoelium* sp.	Cesspit	Le Bailly and Bouchet, 2010

	Lugdunensis	Troyes, Place de la Liberation	Roman	*Dicrocoelium* sp., *Entamoeba histolytica*	Cesspit	Le Bailly and Bouchet, 2010; Le Bailly and Bouchet, 2015
	Narbonensis	Villevieille	Roman	*Ascaris* sp.	?	Dufour, 2015
Germany	*Germania Superior*	Ladenburg	1–200 CE	*Ascaris* sp., *Trichuris* sp.	Latrine	Goppelsröder and Sommer, 1996
	Belgica	Belginum	101–300 CE	*Ascaris* sp., *Capillaria* sp., *Taenia* sp.	Pit	Dittmar et al., 2002
	Raetia	Künzing	140–250 CE	*Trichuris trichiura*	Pit	Specht, 1963
Greece	*Achaia*	Ayia Irini	101–300 CE	*Ascaris lumbricoides*, *Trichuris trichiura*	Pelvic soil	Anastasiou et al., 2018
Israel	*Iudaea*	Qumran	100 BCE–68 CE	*Ascaris lumbricoides*, *Dicrocoelium* sp., *Enterobius vermicularis*, *Taenia* sp., *Trichuris trichiura*	Latrine	Harter et al., 2004; Zias et al., 2006
	Iudaea	Silwan	1–100 CE	*Echinococcus granulosus*	Cyst from skeleton	Zias and Mumcuoglu, 1991
	Iudaea	Nahal-Mishmar Valley	160 CE	*Trichuris trichiura*	Coprolites	Witenberg, 1961
	Iudaea	Caesarea	Roman	*Ascaris lumbricoides*, *Diphyllobothrium* sp.	Latrine	Harter, 2003
Italy	*Italia*	Pompeii	100 BCE–79 CE	*Trichuris trichiura*	Sewer	Heirbaut et al., 2011
	Italia	Portus	25–660 CE	*Ascaris* sp., *Fasciola* sp., *Trichuris* sp.	Sediment cores	Dufour, 2015
	Italia	Roma	Roman	*Entamoeba histolytica*	?	Le Bailly and Bouchet, 2015

Netherlands	*Germania Inferior*	Utigeest	101 BCE–300 CE	*Trichuris* sp.	Well	Van Geel et al,. 2003
	Germania Inferior	Alphen on the Rhine	1–100 CE	*Ascaris* sp., *Taenia/Echinococcus* sp., *Trichuris* sp.	Latrine	Kuijper and Turner, 1992
	Germania Inferior	Valkenburg (Roman Army Camp)	1–100 CE	*Ascaris lumbricoides*, *Trichuris trichiura*	Occupation sediment	Jansen and Over, 1966
Switzerland	*Germania Superior*	Augst	1–100 CE	*Ascaris* sp., *Trichuris* sp.	Latrine	Hänggi et al., 1989; Hufschmid and Sütterlin, 1992
	Germania Superior	Eschenz	1–200 CE	*Ascaris lumbricoides* *Trichuris trichiura*	Latrine	Jauch, 1997
	Germania Superior	Vindonissa	30–45 CE	Ascarididae *Trichuris* sp.	Latrines	Le Bailly et al., 2003c
Turkey	*Asia*	Sagalassos	101-500 CE	*Ascaris* sp., *Giardia duodenalis*	Latrine	Williams et al., 2017

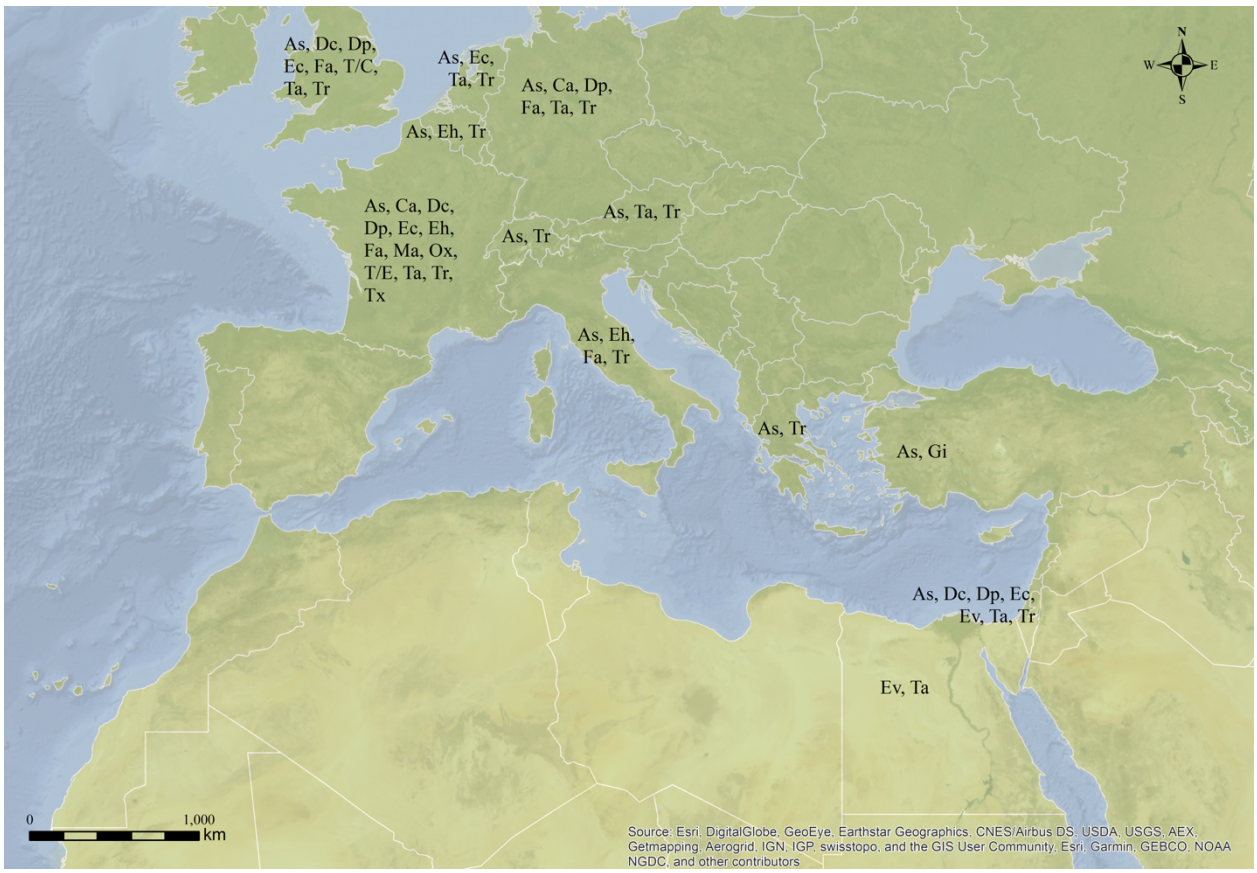

Figure 9: Parasites found at Roman-period sites, mapped onto modern-day countries. As=*Ascaris*, Ca=*Capillaria*, Dc=*Dicrocoelium*, Dp=*Diphyllobothrium*, Ec=*Echinococcus*, Eh=*Entamoeba histolytica*, Ev=*Enterobius vermicularis*, Fa=*Fasciola*, Gi=*Giardia*, Ma=*Macracanthorhynchus*, Ox=*Oxyuris equi*, T/C=*Taenia*/*Capillaria*, T/E=*Taenia*/*Echinococcus*, Ta=*Taenia*, Tr=*Trichuris*, Tx=*Toxocara*.

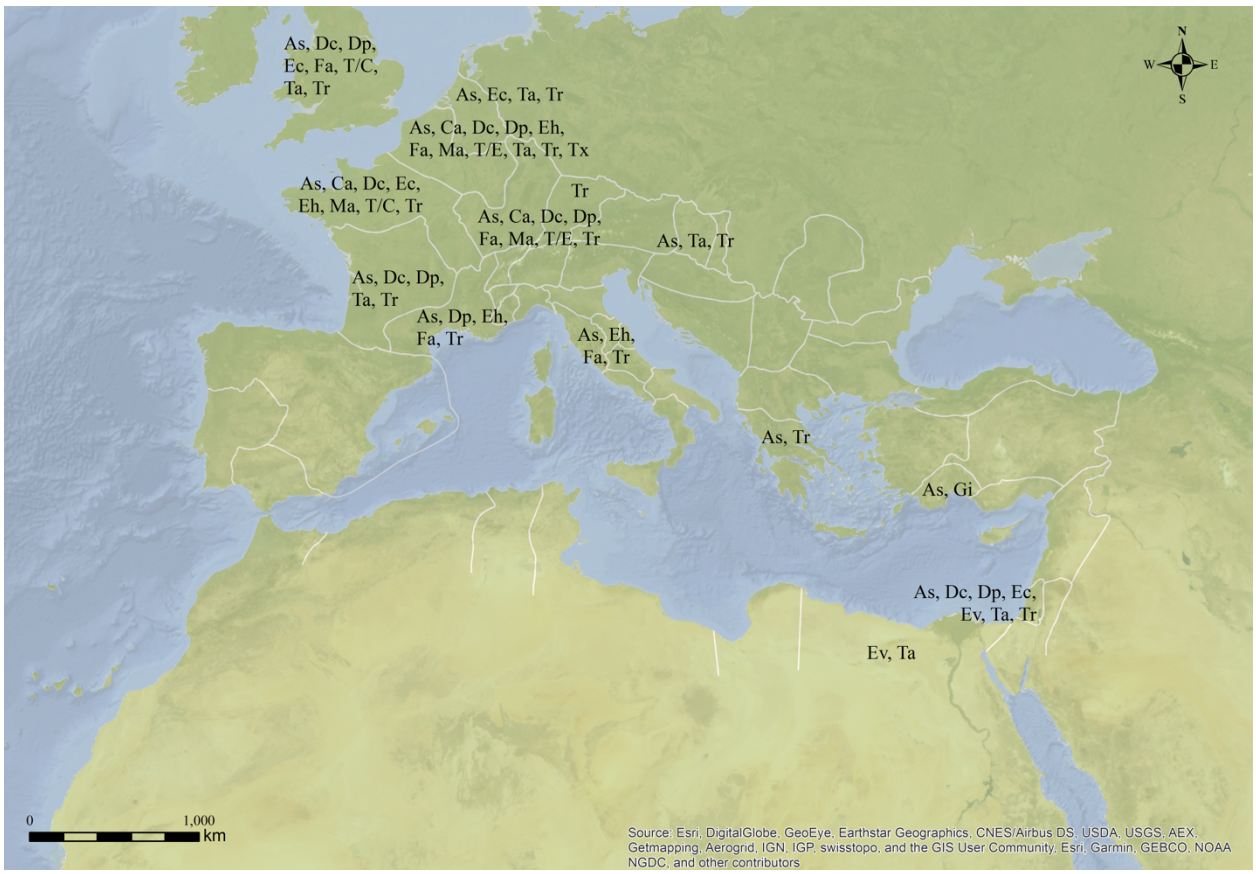

Figure 10: Parasites found at Roman-period sites, mapped onto Roman provinces in 117 CE. Borders of the Roman provinces are from the Digital Atlas of Roman and Medieval Civilizations, Harvard University. As=*Ascaris*, Ca=*Capillaria*, Dc=*Dicrocoelium*, Dp=*Diphyllobothrium*, Ec=*Echinococcus*, Eh=*Entamoeba histolytica*, Ev=*Enterobius vermicularis*, Fa=*Fasciola*, Gi=*Giardia*, Ma=*Macracanthorhynchus*, T/C=*Taenia*/*Capillaria*, T/E=*Taenia*/*Echinococcus*, Ta=*Taenia*, Tr=*Trichuris*, Tx=*Toxocara*.

2.3.6.1 Faecal-Oral Helminths

Whipworm (*Trichuris* sp.) and roundworm (*Ascaris* sp.) have been the most commonly identified parasites in the Roman Empire with whipworm being identified from 34 sites and roundworm from 31 sites. Whipworm has been found in the modern-day countries of Austria, Belgium, Britain, France, Germany, Greece, Israel, Italy, the Netherlands, and Switzerland.

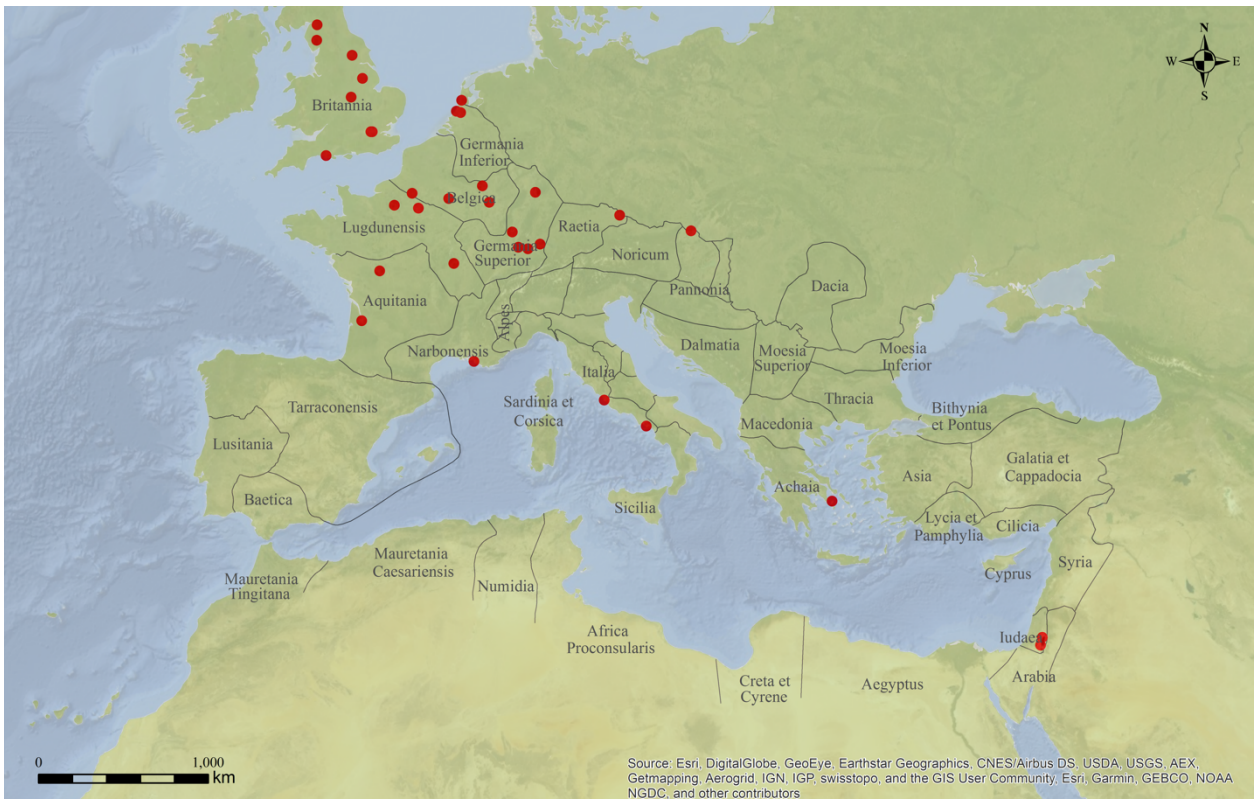

Figure 11: Roman-period sites where whipworm (*Trichuris* sp.) has been found. Sites are marked by red dots and placed in the corresponding Roman province. Roman provincial boundaries (117 CE) are from the Digital Atlas of Roman and Medieval Civilizations, Harvard University.

One quarter of the samples that were found to have whipworm come from Britain, these all correspond to the Roman province of *Britannia*. In London, sediments from Roman period wells (Rouffignac,1985) and ditches (De Moulins, 1990) were found to contain human whipworm. Human whipworm was also identified in occupation layer sediments from the site of Carlisle, Britain (Jones, 1987) and from sediments found within the coffins of individuals buried at Poundbury, Dorset (Jones and Hutchison, 1991). Pike (1968) studied sediment samples from a pit in Owslebury, Britain and found eggs that were identified as either *Trichuris* sp. or *Capillaria* sp. However, it was not known if these parasites were of human or animal origin. In an analysis of sediment from a Roman period sewer in York, Wilson and Rackham (1976) identified *Trichuris* sp. eggs. Similarly in Leicester, eggs of *Trichuris* sp. were identified in sediment from a cesspit (Boyer, 1999). However, they were also uncertain if these eggs were from the human whipworm or an animal species. Eggs from the genus *Trichuris* were also found in samples from a Roman period sewer at the fort of Bearsden, Scotland (Knights et al., 1983). Again, the authors were not sure if these were human whipworm eggs or animal in origin.

Samples from ten sites in France have contained eggs from whipworm. Human-specific whipworm was identified at two sites; the first was pelvic soil from Roman period burials at Bobigny hospital (Rousset et al., 1996); and the second in sediments from coffins of individuals buried in Jaunay-Clan (Dufour et al, 2016). *Trichuris* sp. eggs were identified in pelvic soil from Evreux (Dufour, 2015), based on the context these most likely represent human infection. *Trichuris* sp. were identified in latrine or pit sediment from Beauvais (Dufour, 2015), Bordeaux (Sireix, 2008), Horbourg-Wihr (Dufour, 2015), and Reims (Le Bailly et al., 2003b). *Trichuris* sp. were also identified in occupation sediments from Marseille and samples from Metz and Autun (Dufour, 2015).

In Austria, one case of whipworm comes from the fill of a cesspit at the site of Carnuntum (Petznek, 2018). In the Netherlands, human whipworm was identified in sediment samples taken from the fort of Valkenburg on the Rhine (Jansen and Over, 1966). *Trichuris* sp. eggs were identified from a latrine at the site of Alphen on the Rhine (Kuijper and Turner, 1992) and in sediment from a well at Utigeest (Van Geel et al., 2003). Human whipworm has also been found in the intestines of a bog body from the Netherlands (Searcey et al., 2013). The body, known as Zweeloo woman, is that of a female between 35 and 50 years old and though her body was found just outside the border of the Roman Empire she was presumed to have lived in a Roman settlement nearby, that has not yet been found (Searcey et al., 2013).

In Switzerland, whipworm has been identified in sediment from latrines at three different sites; Augst (Hänggi et al., 1989), Eschenz (Jauch, 1997), and Vindonissa (Le Bailly et al., 2003c).

In Germany, human whipworm (*T. trichiura*) was identified in pit sediment from Künzing (Specht, 1963) and *Trichuris* sp. was identified in a latrine from Ladenburg (Goppelsröder and Sommer, 1996).

At two of three sites studied in Roman Italy whipworm eggs have been found. It is not clear if the sample from Roma, the only sample without whipworm, was studied for both helminths and protozoa or if it was only analysed for protozoa using ELISA. Whipworm has also been found at Pompeii. In samples taken from sewers and drains human whipworm was identified and presumed to have infected Romans living in the city (Heirbaut et al., 2011). *Trichuris* sp. eggs were also found in sediment cores from Portus, these core samples date between 25–660 CE, though it is unclear if parasites in these cores are from humans infected in the city or animals (Dufour, 2015). They do, however, show what taxa of parasites were present in the community.

In Israel, human whipworm has been identified from both coprolites and latrine sediments. Witenberg (1961) analysed coprolites from a Roman period site in the Nahal-Mishmar Valley and identified human whipworm. Whipworm was also identified in latrine samples taken from the site of Qumran (Harter et al., 2004; Zias et al., 2006).

In Greece, human whipworm was recently identified in pelvic soil from burials at the site of Ayia Irini on the island of Kea (Anastasiou et al., 2018). One of the ten individuals studied was infected with whipworm.

Finally, in Belgium we also have evidence for whipworm from one site, the Roman *vicus* (village) at Arlon (Defgnée et al., 2008). The *Trichuris* sp. eggs came from vats and pipes that were thought to be part of a fuller's shop thus these eggs could very likely be from animals. Roman texts suggest that human urine was collected to be reused in the process of fulling (Flohr and Wilson, 2011) thus it is possible that these eggs come from humans if faecal material was also present.

Human roundworm (*Ascaris lumbricoides*) is the second most common parasite found in the Roman Empire. It has been found at Roman period sites in Austria, Belgium, Britain, France, Germany, Greece, Israel, Italy, the Netherlands, Switzerland, and Turkey. The geographical distribution of roundworm (Figure 12) is very similar to that of whipworm (Figure 11).

Similar to whipworm, roundworm has been identified in sediment samples taken from sites throughout Britain. Roundworm was identified alongside human whipworm in sediments from Hibernia Wharf, London (Rouffignac, 1985), in the coffin sediments from Poundbury, Dorset (Jones, 1987), and in the sediment samples from the site of Carlisle (Jones and Hutchison, 1991). At Owslebury, Pike (1968) identified eggs from *Ascaris* sp. alongside whipworm. In the sewer samples from the Church Street Sewer in York and Bearsden in Scotland, roundworm was found with whipworm (Wilson and Rackham, 1976; Knights et al., 1983). Finally, roundworm was also found alongside whipworm in the cesspit sediment from Leicester (Boyer, 1999) and the pit sediment from Ambleside (Jones, 1985). Thus, whipworm was found at all sites in Britain where roundworm was identified, though whipworm has been identified in the absence of roundworm at two sites (Table 8).

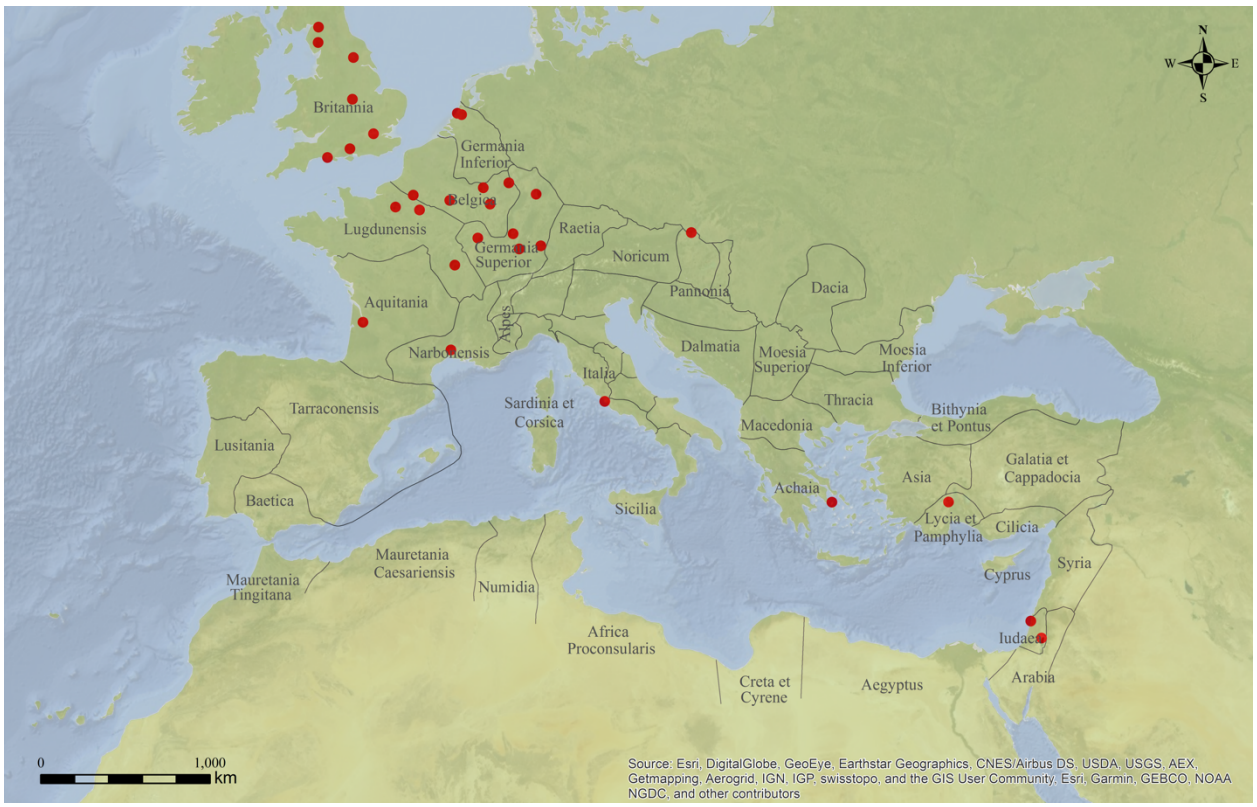

Figure 12: Roman-period sites where roundworm (*Ascaris* sp.) has been found. Sites are marked by red dots and placed in the corresponding Roman province. Roman provincial boundaries (117 CE) are from the Digital Atlas of Roman and Medieval Civilizations, Harvard University.

In France, human roundworm was found alongside whipworm in pelvic soil from burials at Bobigny hospital (Rousset et al., 1996) and Evreux (Dufour, 2015). *Ascaris* sp. eggs were also found in pits, latrines, and sediment samples from Andilly-en-Bassigny, Autun, Beauvais, Bordeaux, Horbourg-Wihr, Metz, Reims, and Villevieille. Andilly-en-Bassigny and Villevieille are the only two sites where roundworm was found in the absence of whipworm. Similarly, there are two sites that had evidence for whipworm in the absence of roundworm, Marseille and Jaunay-Clan.

Roundworm eggs have been found at two Roman sites in Germany. They were found in pit sediment from Belginum (Dittmar et al., 2002) and cesspit sediment from Ladenburg (Goppelsröder and Sommer, 1996).

In the Netherlands, roundworm was identified in the two samples where whipworm was found; the sediments from the Valkenburg Army camp (Jansen and Over, 1966) and latrine sediment from Alphen on the Rhine (Kuijper and Turner, 1992). The samples from the intestines of Zweeloo woman also contained roundworm eggs (Searcey et al., 2013).

In Israel, human roundworm has been found in sediment from a latrine at the site of Caesarea Maritima (Harter, 2003). It was also found in the latrine sediments studied from Qumran which also contained eggs of whipworm (Harter et al., 2004; Zias et al., 2006).

In Switzerland, roundworm eggs were found alongside whipworm eggs in latrines at Augst (Hänggi et al., 1989) and Eschenz (Jauch, 1997). In the latrine from Vindonissa where whipworm eggs were identified, eggs from the family *Ascarididae* were found but they could not be identified more specifically (Le Bailly et al,. 2003c). However, based on the other latrines studied in Switzerland and northern Europe it seems quite likely that these could be eggs from *Ascaris* sp.

In Belgium, eggs of *Ascaris* sp. were found in the vats and pipes that were thought to be part of the fuller's workshop in the *vicus* at Arlon (Defgnée et al., 2008). Again these eggs could be from infected animals or humans.

Human roundworm has been identified at one site in Austria. The site of Carnuntum, from which a latrine and a sewer were sampled and found to contain roundworm eggs (Aspöck et al., 2011).

Pelvic soil from one of the ten individuals studied from the site of Ayia Irini in Kea was found to contain roundworm eggs (Anastasiou et al., 2018). This was a different individual than the one who was found to be infected with whipworm.

In Turkey, roundworm eggs were found in sediment samples collected from a communal latrine in the city of Sagalassos (Williams et al., 2017). Though the latrine was later reused as a stable, the samples from these layers were confirmed to contain human faecal material using faecal lipid biomarkers including 5β stanols which can differentiate omnivore and herbivore faeces (Bull et al., 2002).

High levels of roundworm and whipworm in the Roman Empire may point towards poor hygiene and sanitation as these parasites are spread by the faecal-oral route. The co-occurrence of these two parasites in samples from Roman sites is very apparent. There are few sites where parasite eggs can be attributed to a single individual, which does not allow for us to determine if individuals living in these communities were co-infected or if both parasites existed but many individuals were infected with one or the other. The only site where this evidence exists is Ayia Irini in Greece where roundworm eggs were found alone in one individual and whipworm eggs were found alone in another.

2.3.6.2 Zoonotic Cestodes

Taenia tapeworm has been identified in seven samples studied from Roman period sites. There are three species of *Taenia* tapeworm that are known to infect humans; *Taenia saginata*, *Taenia solium*, and *Taenia asiatica*. Infection with *T. saginata* primarily comes from eating beef, *T. solium* from pork, and *T. asiatica* which is primarily found in Asia is acquired by eating pork. These three species of *Taenia* cannot be distinguished based on morphological differences of eggs seen under the microscope, therefore they are commonly identified in archaeological samples as *Taenia* sp. without identification down to the species level. In order to differentiate a taeniid egg, DNA analysis would be necessary and the cost of this type of analysis is often not realistic in many projects. The seven sites from the Roman Empire that had samples positive for *Taenia* sp. come from Austria, Britain, Egypt, France, Germany, Israel, and the Netherlands (Figure 13).

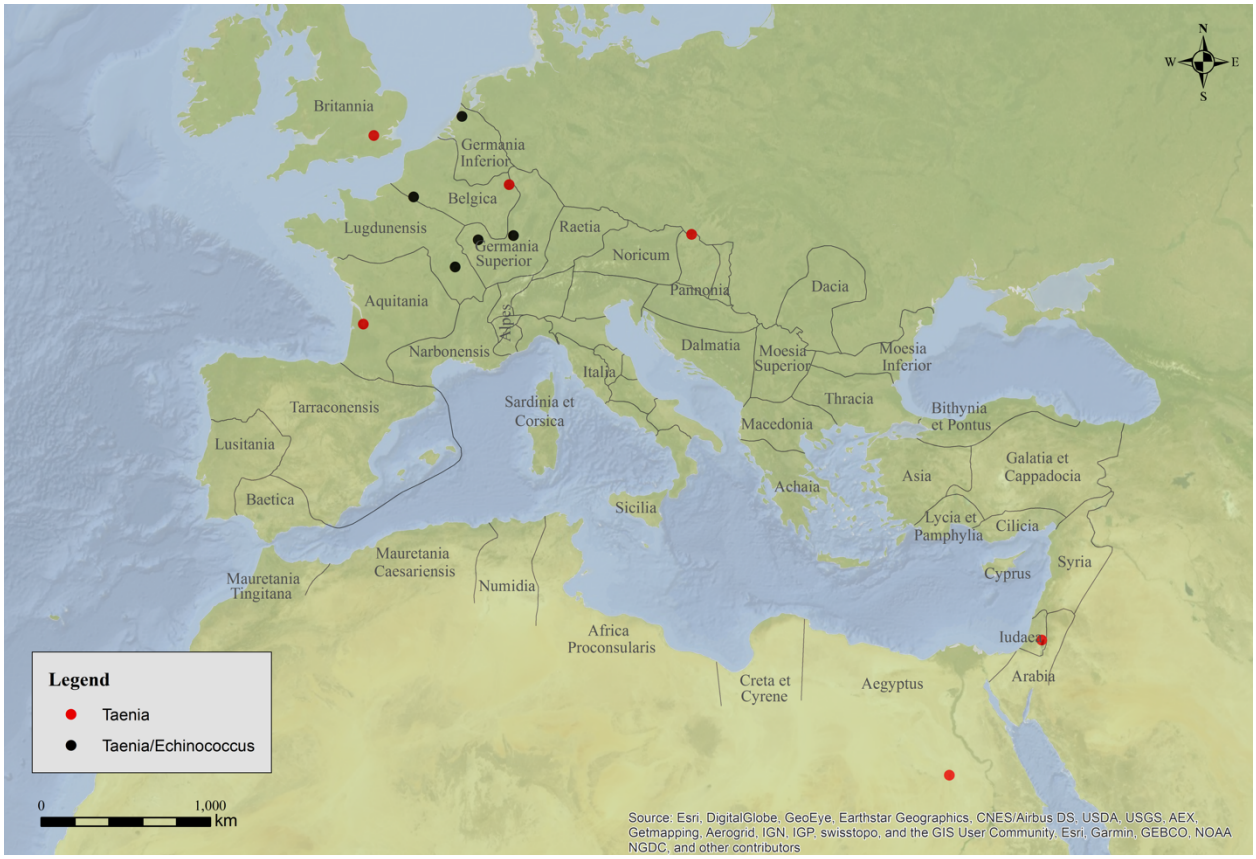

Figure 13: Roman-period sites where beef/pork tapeworm (*Taenia* sp.) has been found. Red dots are sites where *Taenia* sp. were identified and black dots are where eggs were identified as *Taenia* sp. or *Echinococcus* sp. Sites are placed in the corresponding Roman province. Roman provincial boundaries (117 CE) are from the Digital Atlas of Roman and Medieval Civilizations, Harvard University.

In addition, it is often not possible to distinguish the various Taeniid eggs (including *Taenia* spp. and *Echinococcus* spp.) under the microscope (Craig et al., 1986; Dufour, 2015).

As such there are an additional four sites where eggs attributed to *Taenia*/*Echinococcus* sp. have been found in France, and one in Germany (Figure 13).

In Austria, *Taenia* sp. eggs were found in the fill of a 2nd c. CE cesspit (Petznek, 2018). In Britain, Rouffignac (1985) identified *Taenia* sp. tapeworm in the sediment samples from Hibernia Wharf, London. In Egypt, *Taenia* sp. eggs were identified from samples taken from the abdomen of a mummy from the site of El-Deir (Le Bailly et al., 2010). *Taenia* tapeworm eggs were also identified in latrine sediments from the site of Qumran, Israel dated between 100 BCE and 68 CE (Harter et al., 2004; Zias et al., 2006). *Taenia* sp. eggs were found in sediment samples from a latrine in the Netherlands (Kuijper and Turner, 1992) and pit sediment from Germany (Dittmar et al., 2002).

In France, *Taenia* sp. eggs were found in sediment from a latrine and drain from Bordeaux (Sireix, 2008). Eggs belonging to either *Taenia* sp. or *Echinococcus* sp. were found in sediment samples from four other sites in France; Andilly-en-Bassigny, Autun, Beauvais, and Horbourg-Wihr (Dufour, 2015).

Eggs from *Taenia* or *Echinococcus* sp. were identified in latrine sediment from Germany from the site of Alphen on the Rhine (Kuijper and Turner, 1992).

Infection with *Taenia* tapeworms is acquired through ingestion of undercooked pork or beef. Commonly agreed upon staples of the Roman diet included cereals, olive oil, wine, and dry legumes which likely made up a majority of the caloric intake for lower class individuals (Garnsey, 1999a). However, pork and beef were known to be eaten in varying quantities across the Roman Empire and this would vary based on social status and region (King, 1999; Cool, 2006). It is also known that pork was not always cooked but was also eaten salted and dried (Garnsey, 1999a), this could have put individuals at risk for infection with *Taenia solium*.

Fish tapeworm (*Diphyllobothrium* sp., recently renamed *Dibothriocephalus* sp.) has been found at six Roman period sites; in Britain, France, and Israel (Figure 14). There are many species of fish tapeworm with around fourteen species in the genus of *Diphyllobothrium* known to infect humans (Scholz et al., 2009; Garcia, 2016); and the eggs from different species can be quite difficult to distinguish from one another based on morphology. For this reason, many diagnoses in the archaeological record are only genus-level identifications, or the species-level identification has been made based on the most common species found in the corresponding region.

Figure 14: Roman-period sites where fish tapeworm (*Diphyllobothrium* sp.) has been found. Sites are marked by red dots and placed in the corresponding Roman province. Roman provincial boundaries (117 CE) are from the Digital Atlas of Roman and Medieval Civilizations, Harvard University.

In Britain, Rouffignac (1985) identified *Diphyllobothrium latum* in sediment samples from a Roman well at Hibernia Wharf, London. The approach used for determining the species of the eggs retrieved was not described.

In a review of fish tapeworm published by Le Bailly and Bouchet (2013) including previous publications identifying fish tapeworm in the past, as well as listing new results from their lab, *Diphyllobothrium* sp. was identified from the Roman period site of Mikelauen-Zilo. The eggs were found in sediment samples from occupation layers at the site. As mentioned previously, the eggs of the lancet liver fluke were also found at Mikelauen-Zilo and published separately. *Diphyllobothrium* sp. were also identified in occupation sediment from Marseille (Harter-Lailheugue, 2006), pits from Horbourg-Wihr, and an unknown sample type from Metz (Dufour, 2015). This evidence all comes from France.

In Israel, *Diphyllobothrium* sp. was found in sediments from latrines at the site of Caesarea Maritima (Harter, 2003). The latrine samples that were taken came from residential latrines outside the city centre.

Fish tapeworm is acquired by eating raw or undercooked fish. There are four common species of fish tapeworm known to infect humans (*D. latum, D. nihonkaiense, D. pacificus,*

and *D. dendriticum*); all are parasites of freshwater fish except for *D. pacificus* which is found in marine fish in S. America and Australia. The common human-infecting species present in Europe and the Mediterranean are all acquired from eating freshwater fish. The proportion of freshwater and marine fish that contributed to Roman diets likely varied across the Empire. We might expect that marine fish were more commonly eaten in the Mediterranean basin which may have resulted in fewer infections in these regions. We know that fish was part of the Roman diet and was traded extensively in the Roman Empire in the form of *garum* (fish sauce) or *salsamentum* (salted fish) (Curtis, 1991; Marzano, 2018). Fish in both of these preparations is uncooked and could have been a source of infection. Further evidence for fish tapeworm in the Roman Empire may allow us to determine if fish tapeworm infection was primarily from local fish or widespread trade of preserved fish to areas without regular access to freshwater fish.

2.3.6.3 Zoonotic Trematodes

Fasciola liver fluke has been found at eight sites from the Roman Empire. These are located in Britain, France, and Italy (Figure 15). These samples are a mix of sediment from latrines, cesspits, and occupation layer sediment; as such it is difficult to tie any of these eggs specifically to human infections as eggs could be from animal faeces that were dumped in latrines or contaminated settlement areas. However, if animal faeces were found around settlement areas it is likely that cases of pseudoparasitism existed if humans ingested *Fasciola* sp. eggs that contaminated food or water or ate raw liver from sheep or cattle. Similarly, true infections could have occurred if aquatic plants carrying the encysted metacercariae were eaten, especially from bodies of freshwater near grazing sheep or cattle.

In Britain, Jones and Hutchison (1991) recovered eggs of a parasite from the genus *Fasciola*, but were unable to determine the species. These eggs came from sediment samples taken from the site of Carlisle, however, a mix of animal and human parasites were found in these samples so they could not determine if the *Fasciola* sp. eggs were of human or animal origin. *Fasciola* sp. eggs were also identified in sediment from a cesspit in Leicester (Boyer, 1999).

Figure 15: Roman-period sites where *Fasciola* sp. liver fluke has been found. Sites are marked by red dots and placed in the corresponding Roman province. Roman provincial boundaries (117 CE) are from the Digital Atlas of Roman and Medieval Civilizations, Harvard University.

Most evidence for *Fasciola* liver fluke in the Roman Empire comes from France. *Fasciola* sp. eggs have been found in pits, latrines, and occupation sediments from Andilly-en-Bassigny, Horbourg-Wihr, Marseille, Metz, and Reims (Le Bailly et al., 2003b; Harter-Lailheugue, 2006; Dufour, 2015). However, none of these samples can give convincing evidence for human infection with *Fasciola* sp. though they do indicate that the parasite was present in Roman settlements in France and humans were at risk for infection.

Fasciola hepatica is a zoonotic disease that occurs when humans are living in close proximity to the natural hosts of the parasite including sheep, goats, and cattle (Garcia, 2016: 499). Infection is typically a result of consumption of contaminated water, or ingestion of metacercariae encysted on aquatic plants such as watercress and water chestnuts (Vennervald, 2018). Therefore, it is especially prevalent in areas where animal faeces are used as a form of fertilizer. Today, *Fasciola hepatica* can be found in most countries around the world. In the past, we would expect it would have been most often found in populations living closely with herded animals especially sheep. It is known from archaeological and historical evidence that sheep and cattle were herded and eaten in Roman Britain and Gaul, both of which could have been sources of *F. hepatica* infection (King, 1999; Cool, 2006). While sheep and goats were

not always a major source of meat they were kept for wool/hair and cheese (Garnsey, 1999a). Even if these animals did not constitute a large portion of the diet, if they were kept in proximity to residences they could have posed a risk for zoonotic infection with *F. hepatica*.

The lancet liver fluke (*Dicrocoelium dendriticum*) is a trematode that infects humans after ingestion of ants, often accidently when ants are on plants being eaten (Garcia, 2016:505). The lancet liver fluke has been found at eight Roman sites, located in Britain, France, and Israel (Figure 16). Five out of eight of these sites are in France, many of which were reported in a review of dicrocoeliosis in the past (Le Bailly and Bouchet, 2010). All evidence for *Dicrocoelium* sp. in France has come from sediment samples from latrines, cesspits, or occupation layers. This evidence comes from sites in Beauvais, Horbourg-Wihr, Mikelauen-Zilo, Reims, and Troyes (Le Bailly and Bouchet, 2010; Dufour, 2015).

Figure 16: Roman-period sites where lancet liver fluke (*Dicrocoelium* sp.) has been found. Sites are marked by red dots and placed in the corresponding Roman province. Roman provincial boundaries (117 CE) are from the Digital Atlas of Roman and Medieval Civilizations, Harvard University.

Aside from France, *Dicrocoelium* sp. eggs have been found at two sites in Britain. Pike (1968) identified eggs of *Dicrocoelium* sp. in the pit sediments studied from the site of Owslebury; these were found alongside the eggs of whipworm and roundworm. *Dicrocoelium dendriticum* eggs were found in ditch sediment from London (15–35 Copthall Ave.) (De Moulins, 1990).

The lancet liver fluke was also found in the bog body of Zweeloo woman from the Netherlands (Searcey et al., 2013). However, rather than finding the eggs in the intestinal wash, as was the case for whipworm and roundworm, the eggs of the lancet liver fluke were found in thin sections of preserved liver tissue, indicating true infection.

In Israel, the lancet liver fluke was identified in the samples from the latrine at Qumran, alongside whipworm and roundworm (Zias et al., 2006).

Apart from Israel, the lancet liver fluke has not yet been found from Roman sites east of France which includes most of the Mediterranean basin. However, with only this limited amount of data it is difficult to interpret if this is a sampling bias, as relatively more sites have been studied in France and Britain, or if the lancet liver fluke was truly absent in other parts of the Roman Empire. The lancet liver fluke has been found at Neolithic sites in Germany (Le Bailly, 2005), Switzerland (Dommelier-Espejo, 2001), and Spain (Maicher et al., 2017), and as far south as Sudan in the Bronze Age (Harter, 2003), which indicates that it was already present in these areas before their occupation by the Romans, and quite possibly could have persisted throughout the Roman Empire.

The geographical distribution of the lancet liver fluke in the Roman period (Figure 16) is very similar to *Fasciola* liver fluke (Figure 15). This may be a result of similar animal hosts for these two species and the potential for eggs to be found in human faeces as a result of similar cultural practices (e.g. eating liver).

2.3.6.4 Protozoa

Protozoa such as *Giardia duodenalis* and *Entamoeba histolytica* that are known to cause dysentery or severe diarrhoea have also been found in Roman period samples. The cysts of these protozoa are smaller than helminth eggs and do not preserve well in archaeological samples so are difficult to find using light microscopy. Therefore, they are often detected using immunological methods, particularly direct-antigen ELISA, which can detect preserved antigens specific to these protozoa in cases where the cysts can longer be visualized.

Figure 17: Roman-period sites where *Giardia duodenalis* (red dots) and *Entamoeba histolytica* (blue dots) have been found. Sites are placed in the corresponding Roman province. Roman provincial boundaries (117 CE) are from the Digital Atlas of Roman and Medieval Civilizations, Harvard University.

Entamoeba histolytica has been found in six Roman period sites. These samples have come from Belgium, France, and Italy. In their review of *Entamoeba histolytica* in the past, Le Bailly and Bouchet (2015) reported detection of *E. histolytica* using ELISA performed on latrine samples taken from the site of Mageroy, Belgium; from sediment taken from the site of Roma, Italy; and from samples taken from a pit at the site of Lisses and a latrine at Troyes, France. Also in France, Le Bailly and Bouchet (2006) found *E. histolytica* in cesspit samples taken from the site of Lattes. Gonçalves et al. (2004) found *E. histolytica* in cesspit samples from the Roman villa La Gramiere.

In Israel, Witenberg (1961) reported finding *E. histolytica* in samples using microscopic analysis of coprolites taken from the Nahal-Mishmar Valley. This is an exceptional finding because the cysts of these organisms are smaller than helminth eggs and do not preserve as well, so are not typically seen with microscopy. No pictures of the protozoa were included in the report so its presence cannot be confirmed. Furthermore, the cysts of *Entamoeba histolytica* are indistinguishable from the non-pathogenic *Entamoeba dispar* using microscopy, bringing these results further into question. He also reported finding *Giardia duodenalis,* but again without pictures this finding cannot be confirmed.

Currently, there is evidence for *Giardia duodenalis* from one site in the Roman Empire. Sediment samples from layers of a communal latrine at the site of Sagalassos tested positive for *G. duodenalis* using ELISA (Williams et al., 2017). These layers also contained roundworm eggs.

Malaria is an intracellular parasite that must live in the red blood cells of its host in order to survive; as such it cannot be detected in human faecal material. The methods used to detect the malaria parasite (*Plasmodium* spp.) are very different from intestinal parasites. Pathogen DNA extracted from dental pulp of skeletons excavated from Roman period sites has been used to confirm the presence of *Plasmodium falciparum* infection in a few individuals from the Imperial Roman period in Italy. Confirmed cases of *Plasmodium falciparum* come from one individual buried in the cemetery of Vagnari (Marciniak et al., 2016), one individual from Velia (Marciniak et al. 2016), and one infant from Lugnano (Sallares and Gomzi, 2001).

Finally, *Plasmodium falciparum* alongside the protozoa causing toxoplasmosis was found in an Egyptian mummy using aDNA analysis of muscle tissue (Khairat et al., 2013). When humans are infected with toxoplasmosis, through the ingestion of oocysts or raw infected meat, the protozoa proliferate in cells and can remain in inactive form in neural and muscle tissue for the entire life of the host. This is usually not an issue for adults but can cause severe symptoms in neonates infected in utero and immunocompromised individuals. It is not surprising that this is the only case so far identified in the Roman Empire because *Toxoplasma gondii* causes tissue infection and does not release any oocysts in human faeces; therefore the only way to diagnose it is by looking at preserved tissue. Furthermore, aDNA analysis has not currently been used to study any parasites in the Roman Empire except this case of toxoplasmosis and the cases of malaria previously discussed.

*2.3.6.5 Other Helminths (*Capillaria *sp.,* Echinococcus *sp.,* Macracanthorhynchus *sp*., *Pinworm,* Toxocara *sp.)*

Capillaria sp. worm has been identified from a few sites in the Roman Empire. These sites are mostly concentrated in modern-day France. The eggs have been found at six sites in total, five from France and one from Germany (Figure 18).

The only convincing evidence for human infection comes from the site of Amiens in France where eggs of *Capillaria hepatica* were identified in the wall of a hydatid cyst from an adolescent living sometime during the 3rd–4th c. CE (Mowlavi et al., 2014). The eggs

preserved in a cyst indicate that this individual had a co-infection with *Echinococcus granulosus* (hydatidosis) and *Capillaria hepatica.*

Capillaria sp. eggs were also identified in a number of sediment samples from France. These include latrine and pit sediment from Beauvais and Horbourg-Wihr and unknown sample types from Autun and Metz (Dufour, 2015). In these cases it is impossible to know if eggs were from humans infected with *Capillaria* or if the eggs were from infected rodents whose faeces were present in these samples. Similarly, eggs could be found in human faeces in the absence of true infection in cases of pseudoparasitism.

Figure 18: Roman-period sites where *Capillaria* sp. has been identified. Sites are marked by red dots and placed in the corresponding Roman province. Roman provincial boundaries (117 CE) are from the Digital Atlas of Roman and Medieval Civilizations, Harvard University.

Capillaria sp. eggs were also found at one site in Germany. These eggs were found in pit sediment from the site of Belginum (Dittmar et al., 2002). These were found alongside *Taenia* sp. and roundworm, two species that were likely from human infections.

Other intestinal helminths that have been found from Roman period samples are hydatid disease (*Echinococcus granulosus*), pinworm (*Enterobius vermicularis*), and *Toxocara* sp. These parasites have only been found in one or two archaeological samples in the Roman Empire thus far.

Figure 19: Roman-period sites where *Echinococcus* sp., pinworm (*Enterobius vermicularis*), *Maracanthorhynchus* sp., or *Toxocara* sp. have been identified. Sites are placed in the corresponding Roman province. Roman provincial boundaries (117 CE) are from the Digital Atlas of Roman and Medieval Civilizations, Harvard University.

Hydatid disease was identified in a Roman period burial from Jerusalem (Zias and Mumcuoglu, 1991). The authors proposed that two calcified cysts found in the skeletonized remains of an adult were from *Echinococcus granulosus,* based on their size and the corresponding collapsed vertebrae. However, due to the infrequency with which hydatid disease causes vertebral collapse in adults and the large number of other conditions that can result in vertebral collapse this finding is by no means conclusive. As mentioned previously, Mowlavi and colleagues (2014) identified two calcified cysts from the thoraco-abdominal cavity of a Roman period burial in Amiens, France as hydatid cysts. Hydatid cysts were also identified in Britain from the skeleton of a female (~45 years old) from the site of Orton Longueville (Wells and Dalls, 1976).

Pinworm has been found in a coprolite from an Egyptian mummy dated to the Roman period (Horne, 2002), and a sediment sample from a latrine at the site of Qumran, Israel (Zias et al., 2006). There are few cases of pinworm in the Old World as a whole, likely due to the delicate nature of the eggs. It has also been found in Germany (Herrmann, 1985), Greenland (Reinhard et al., 2016), Czech Republic (Bartošová et al., 2011), Iran (Nezamabadi et al.,

2013), Spain (Maicher et al., 2017), and Sudan (Harter, 2003) in other time periods. Recent ancient DNA analysis has found evidence for pinworm in the absence of preserved eggs from Denmark (11th c. CE) and the Netherlands (14th–19th c. CE) (Søe et al., 2018). Today, pinworm is the most common helminth infection in Western Europe and it is probable that the prevalence was also quite high in the past even though it is not always found in archaeological samples. One reason for the lack of evidence for pinworm in the past is due to the delicate nature of pinworm eggs; pinworm eggs have a very thin eggshell and are thus more susceptible to degradation especially in poor soil conditions (Bouchet et al., 2003).

Macracanthorhynchus sp. eggs were identified in sediment samples from three sites in Roman France. These were sediment samples from pits, latrines, ditches, and occupation layers from the sites of Autun, Beauvais, and Horbourg-Wihr (Dufour, 2015). Though *Macracanthorhynchus* sp. can cause infection in humans it is much more common in animals (Garcia, 2016), and the sample types studied do not allow for us to definitively say if these eggs came from the faeces of humans or other animals. *Toxocara* sp. eggs were also reported by Dufour (2015) in samples from the site of Metz.

2.3.7 Parasites in the Medieval Period

Currently, there is a larger amount of comparative evidence for parasites in Europe, the Middle East, and North Africa after the Roman period compared to time periods prior to the Roman period. Again, the Medieval period is defined differently depending on the region. For this work I have included sites identified as Medieval by authors and sites dated between the end of the Roman period up until the early modern period, which starts after the 15th c. CE in Europe and the Middle East. A minimum of 18 taxa have been found in Medieval period Europe, Middle East and North Africa. These include *Capillaria* sp., *Dicrocoelium* sp. liver fluke, *Echinococcus* sp., *Entamoeba histolytica, Fasciola* sp. liver fluke, fish tapeworm, *Giardia duodenalis, Hymenolepis* sp., *Leishmania donovani*, pinworm, roundworm, *Schistosoma haematobium*, *Schistosoma mansoni*, *Taenia* sp., *Taenia saginata*, *Toxocara* sp., and whipworm. Similar to the Roman period, archaeological sites from Britain and France have been the most extensively studied for parasites in the Medieval period but evidence also comes from Austria, Belgium, Cyprus, Czech Republic, Germany, Israel, Italy, Jordan, Netherlands, Portugal, Spain, Sudan, and Switzerland.

The most common helminths found in the Medieval period are roundworm (*Ascaris* sp.) and whipworm (*Trichuris* sp.) followed by the lancet liver fluke (*Dicrocoelium* sp.), beef/pork tapeworm (*Taenia* sp.), fish tapeworm (*Diphyllobothrium* sp.), and *Fasciola* liver fluke. The protozoa *Entamoeba histolytica* and *Giardia duodenalis* have also been found.

Other less common parasites that have been found include *Echinococcus* sp., *Hymenolepis* sp., leishmaniasis, pinworm (*Enterobius vermicularis*), schistosomiasis, and *Toxocara* sp.

Table 9: Parasites found at Medieval period sites.

Country	Site	Date	Parasites	Sample Type	Citation
Austria	Am Hof, Wien	Medieval	*Ascaris lumbricoides*	Sewer	Auer and Aspöck, 2014
Belgium	Namur	9th–15th c. CE	*Ascaris* sp., *Diphyllobothrium* sp., *Entamoeba histolytica, Fasciola hepatica*, *Taenia* sp., *Trichuris* sp.	Cesspit	Gonçalves et al., 2004; da Rocha et al., 2006
	Nivelles	10th–13th c. CE	*Ascaris lumbricoides*, *Trichuris trichiura*	Coprolites	Rácz et al., 2015
	Malines Prison	14th c. CE	*Ascaris* sp., *Trichuris* sp.	Cesspits	Troubleyn et al., 2009
	Bruges	14th–15th c. CE	*Ascaris* sp., *Trichuris* sp.	Cesspits	Deforce et al., 2010
	Raversijde	16th c. CE	*Ascaris* sp., *Capillaria* sp., *Trichuris* sp.	Latrines	Fernandes et al., 2005
Britain	London	600–871 CE	*Ascaris* sp., *Taenia* sp., *Trichuris trichiura*	Pit	Tibensky and Sidell, 2003
	Lincoln, Waterside NW and Woolworth's	10th–13th c. CE	*Ascaris* sp., *Trichuris sp.*	Occupation sediment	Carrott et al., 1995
	York (Bedern)	10th–14th c. CE	*Ascaris* sp., *Trichuris* sp.	Latrine	Hall et al., 1983
	York, 16–22 Coppergate	10th–14th c. CE	*Ascaris* sp., *Trichuris* sp.	Pit	Hall et al., 1983
	York	1000 CE	*Ascaris* sp., *Taenia* sp., *Trichuris trichiura*	Coprolite	Flammer et al., 2018
	Winchester	11th–12th c. CE	*Ascaris* sp., *Dicrocoelium dendriticum*, *Trichuris* sp.	Pit	Taylor, 1955; Pike and Biddle, 1966
	London, 15–35 Copthall Ave	11th–12th c. CE	*Ascaris lumbricoides*, *Trichuris trichiura*	Ditch	de Moulins, 1990

	Finzels Reach, Bristol	1150–1700 CE	*Ascaris* sp., *Trichuris trichiura*	Waste deposit	Flammer et al., 2018
	Southampton	13th–14th c. CE	*Ascaris* sp., *Trichuris* sp.	Cesspit	Pike, 1975
	York	14th–16th c. CE	*Ascaris* sp., *Trichuris* sp.	Latrine	Jones and Nicholson, 1988
	Worcester	15th c. CE	*Ascaris* sp., *Trichuris* sp.	Latrine	Greig, 1981
	King Richard III	1485 CE	*Ascaris lumbricoides*	Pelvic soil	Mitchell et al., 2013
	Leicester	Medieval	*Ascaris* sp., *Fasciola* sp., *Trichuris* sp.	Cesspit	Boyer, 1999
	London, Hibernia Wharf	Medieval	*Ascaris lumbricoides*, *Diphyllobothrium latum*, *Fasciola hepatica*, *Taenia* sp., *Trichuris trichiura*	Pit	Rouffignac, 1985
Cyprus	Saranda Kolones	1191–1222 CE	*Ascaris lumbricoides, Trichuris trichiura*	Latrine	Anastasiou and Mitchell, 2013
Czech Republic	Breclav-Pohansko	850–950 CE	*Ascaris* sp., *Trichuris trichiura*	Pelvic soil	Flammer et al., 2018
	Chrudim	13th–18th c. CE	*Ascaris lumbricoides*, *Diphyllobothrium latum*, *Enterobius vermicularis*, *Fasciola hepatica*, *Giardia duodenalis*, *Hymenolepis nana*, *Toxocara canis, Trichuris trichiura*	Cesspit	Bartošová et al., 2011
France	Collège de France	4th–5th c. CE	*Dicrocoelium* sp.	Occupation sediment	Le Bailly and Bouchet, 2010
	Mikelauen-Zilo	10th–14th c. CE	*Dicrocoelium* sp.	Occupation sediment	Le Bailly and Bouchet, 2010
	Charavine	11th c. CE	*Dicrocoelium* sp.	Coprolite	Bouchet et al., 2000
	Pineuilh	11th–13th c. CE	*Entamoeba histolytica*, *Giardia duodenalis*	Occupation sediment	Le Bailly and Bouchet, 2006; Le Bailly et al., 2008
	Grand Louvre, Paris	11th–16th c. CE	*Ascaris lumbricoides*, *Dicrocoelium dendriticum*, *Trichuris trichiura*	Latrine	Bouchet, 1995

	Ile de la Cité	12th–15th c. CE	*Dicrocoelium dendriticum, Fasciola hepatica*, *Taenia* sp., *Trichuris* sp.	Cesspit	Bouchet et al., 1989
	Epinal	13th c. CE	*Dicrocoelium* sp.	Latrine	Le Bailly and Bouchet, 2010
	Villiers-le-Bel	14th c. CE	*Dicrocoelium* sp., *Entamoeba histolytica*	Tomb sediment	Le Bailly and Bouchet, 2010; Le Bailly and Bouchet, 2015
	Montbéliard	1450–1550 CE	*Ascaris lumbricoides*, *Dicrocoelium* sp., *Diphyllobothrium latum*, *Schistosoma haematobium*, *Schistosoma mansoni*, *Trichuris trichiura*	Latrine	Bouchet and Paicheler, 1995; Gonçalves et al., 2003; Bouchet et al., 2002; Le Bailly and Bouchet, 2010
	Rue Cardinal Lemoine, Paris	15th c. CE	*Dicrocoelium* sp.	Latrine	Le Bailly and Bouchet, 2010
	Rigny	15th–16th c. CE	*Ascaris* sp., *Dicrocoelium sp.*, *Trichuris* sp.	Latrine	Bouchet et al., 1995b
	Marly-le-Roy	1680–1715 CE	*Ascaris lumbricoides*, *Fasciola hepatica*, *Taenia* sp., *Trichuris trichiura*	Latrine	Bouchet et al., 1998
	Brouage	17th c. CE	*Entamoeba histolytica*	Latrine	Le Bailly and Bouchet, 2015
Germany	Lübeck	1100–1650 CE	*Ascaris* sp., *Diphyllobothrium latum*, *Taenia saginata*, *Trichuris trichiura*	Latrine	Flammer et al., 2018
	Ellwangen-Jagst	1400–1600 CE	*Ascaris* sp., *Trichuris trichiura*	Pelvic soil	Flammer et al., 2018
Israel	Acre	1190–1291 CE	*Ascaris lumbricoides*, *Diphyllobothrium latum*, *Entamoeba histolytica*, *Giardia duodenalis*, *Taenia* sp., *Trichuris trichiura*	Latrine	Mitchell and Tepper, 2007; Mitchell et al., 2008; Mitchell et al., 2011
	Jerusalem	1450–1550 CE	*Ascaris lumbricoides*, *Diphyllobothrium* sp., *Entamoeba histolytica*, *Giardia duodenalis*, *Taenia* sp., *Trichuris trichiura*	Coprolites and cesspit	Yeh et al., 2015

Italy	Piazza Garibaldi	10th–11th c. CE	*Ascaris* sp., *Capillaria* sp., *Dicrocoelium* sp., *Diphyllobothrium* sp., *Taenia*/*Echinococcus* sp., *Trichuris trichiura*	Cesspit	Bosi et al., 2011; Florenzano et al., 2012
Jordan	Gerasa	650–750 CE	*Ascaris lumbricoides*	Latrine	Søe et al., 2018
Netherlands	Utrecht	13th–14th c. CE	*Ascaris lumbricoides, Trichuris trichiura*	Cesspit	Boersema and Jansen, 1975
	Kampen	1350–1425 CE	*Ascaris lumbricoides*, *Diphyllobothrium* sp., *Enterobius vermicularis, Taenia* sp., *Toxocara* sp., *Trichuris trichiura*	Latrine	Søe et al., 2018
Portugal	Santarém	9th-12th c. CE	*Ascaris lumbricoides*, *Trichuris trichiura*	Pelvic soil	Cunha et al., 2017
	Mértola	12th–13th c. CE	*Ascaris* sp.	Latrine	Knorr et al., 2019
Spain	Córdoba	10th–11th c. CE	*Ascaris* sp.	Latrine	Knorr et al., 2019
	Collegiate-Basilica of St. Isidoro	10th–13th c. CE	*Ascaris lumbricoides, Trichuris trichiura*	Mummy	Hidalgo-Argüello et al., 2003
Sudan	Sedeinga	300–1500 CE	*Ascaris lumbricoides*, *Enterobius vermicularis*, *Hymenolepis nana*, *Schistosoma* sp., *Trichuris trichiura*	Pelvic soil	Harter, 2003
	Wadi Halfa	350–550 CE	*Schistosoma mansoni*	Mummy	Miller et al., 1992
	Kulubnarti	550–1500 CE	*Leishmania donovani*, *Schistosoma mansoni*	Mummy	Zink et al., 2006; Hibbs et al., 2011
	Sai Island	1500 CE	*Hymenolepis* sp., *Trichuris trichiura*	Pelvic soil	Harter, 2003
Switzerland	Chevenez	7th–9th c. CE	*Entamoeba histolytica*, *Giardia duodenalis*	Pelvic soil	Le Bailly, 2005; Le Bailly and Bouchet, 2006
	Mummy	Medieval	*Echinococcus granulosus*	Mummy	Baud and Kramer, 1991

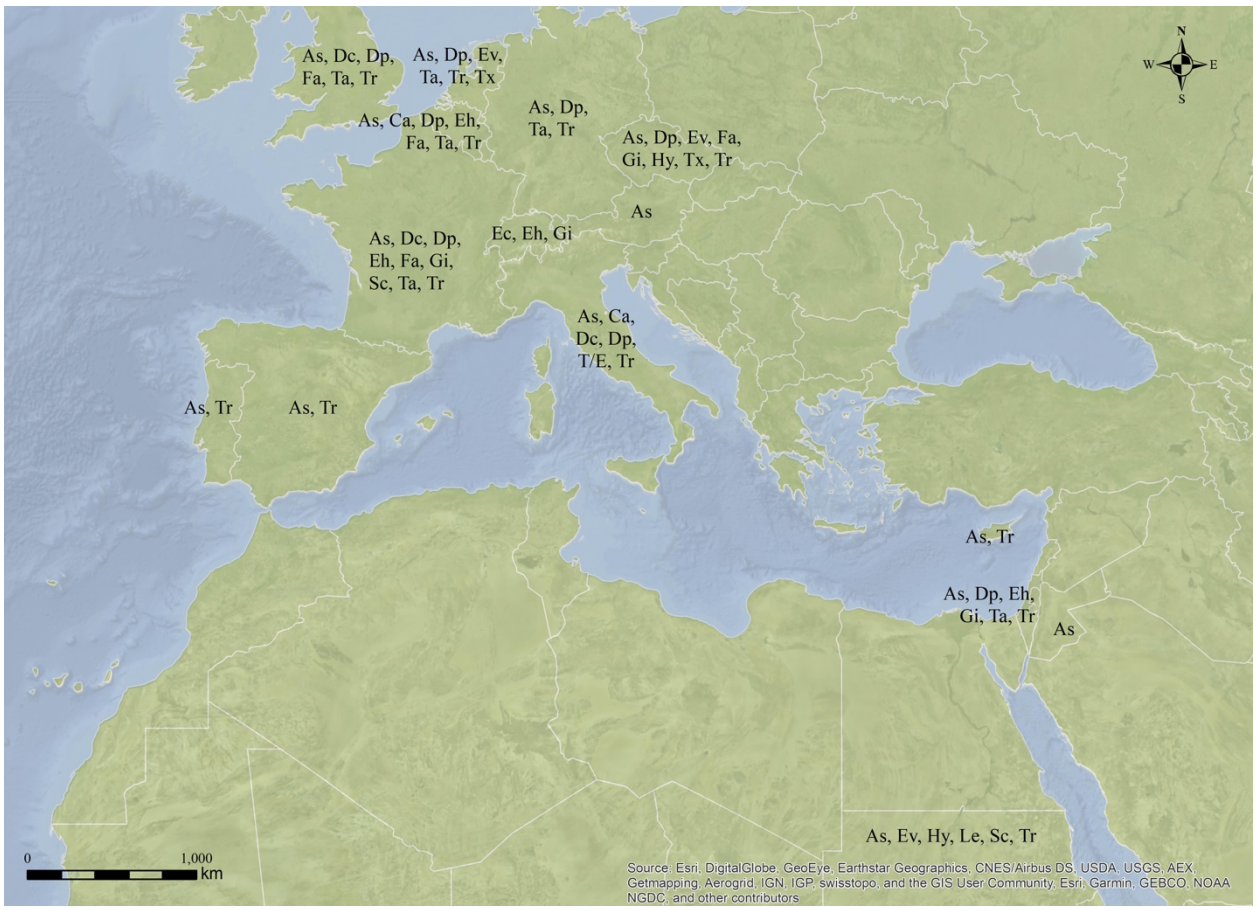

Figure 20: Parasites found at Medieval period sites, mapped onto modern-day countries. As=*Ascaris*, Ca=*Capillaria*, Dc=*Dicrocoelium*, Dp=*Diphyllobothrium*, Ec=*Echinococcus*, Eh=*Entamoeba histolytica*, Ev=*Enterobius vermicularis*, Fa=*Fasciola*, Gi=*Giardia*, Hy=*Hymenolepis*, Le=*Leishmania*, Sc=*Schistosoma*, T/E=*Taenia*/*Echinococcus*, Ta=*Taenia*, Tr=*Trichuris*, Tx=*Toxocara.*

2.3.7.1 Faecal-Oral Helminths

Roundworm and whipworm are the most common parasites found in the Medieval period. Roundworm has been identified at 40 sites and whipworm has been identified at 37 sites in Medieval Europe, Middle East and North Africa. Roundworm has been found in every country that has been studied except for Switzerland. In Austria, human roundworm was found in a sewer at the site of Am Hof, Wien (Auer and Aspöck, 2014). In Belgium, roundworm eggs were identified in cesspits and latrines from Namur, Malines prison, Bruges, Raversijde, and in coprolites from Nivelles (Gonçalves et al., 2003; Rácz et al., 2013).

At the end of the 20th century, sediment samples taken from pits or ditches at Medieval period sites in Britain were commonly studied for botanical remains and parasites as part of the environmental analysis of archaeological excavations. For many of these, the eggs could not be confirmed to be from human parasites. Roundworm eggs were identified in ditches or pit sediments from three sites in London (Rouffignac, 1985; de Moulins, 1990;

Tibensky and Sidell, 2003), one in York (Hall et al., 1983), and in Winchester (Taylor, 1955). They have also been found in cesspit sediments and latrines from two sites in York (Hall et al., 1983; Jones and Nicholson, 1988), one in Southampton (Pike, 1975), in Worcester (Greig, 1981), and in Leicester (Boyer, 1999). Using aDNA Flammer and colleagues (2018) identified *Ascaris* sp. in waste deposits from Bristol and coprolites from York. Finally, the only pelvic soil samples studied in Medieval Britain are from King Richard III and they were found to contain roundworm eggs (Mitchell et al., 2013).

In Cyprus, human roundworm was found in a latrine from the site of Saranda Kolones (Anastasiou and Mitchell, 2013). In the Czech Republic, human roundworm was reported from a cesspit in Chrudim (Bartošová et al., 2011) and from pelvic soil at Breclav-Pohansko, a finding that was confirmed with aDNA studies (Flammer et al., 2018).

In France, human roundworm was identified in latrine samples spanning the 11th–18th centuries CE. These samples come from three sites including the Grand Louvre in Paris, Marly-le-Roy, Montbéliard, and Rigny (Bouchet, 1995; Bouchet et al., 1995b; Bouchet et al., 1998; Gonçalves et al., 2003).

Ancient DNA analysis also confirmed the presence of roundworm in a latrine from Lübeck in Germany and in pelvic soil samples from the site of Ellwangen-Jagst in Germany (Flammer et al., 2018). Microscopic analysis of sediment samples from Crusader latrines in Acre, Israel revealed eggs of roundworm (Mitchell and Tepper, 2007). Roundworm eggs were also found in coprolites and latrine sediment from Mamluk period Jerusalem (Yeh et al., 2015).

In Italy, roundworm eggs have been found in cesspits that were presumed to be used by humans excavated in Piazza Garibaldi, Parma (Bosi et al., 2011; Florenzano et al., 2012).

The only paleoparasitological data from Jordan is from a latrine (650–750 CE) in Gerasa where only roundworm eggs were found using aDNA analysis (Søe et al., 2018).

In the Netherlands, roundworm has been found in cesspits and latrines from two sites which date between the 13th–15th c. CE (Boersema and Jansen, 1975; Søe et al., 2018).

On the Iberian peninsula, roundworm has also been found in pelvic soil from Portugal (Cunha et al., 2017) and a mummy from Spain (Hidalgo-Argüello et al., 2003). It was also recently found in latrines from Córdoba in Spain and Mértola in Portugal (Knorr et al., 2019).

Finally, in Sudan roundworm was been found in pelvic soil collected from burials dating from 300–1500 CE from the site of Sedeinga (Harter, 2003).

Whipworm has been found in samples from 37 sites in Medieval Europe, Middle East, and North Africa. Whipworm is found alongside roundworm in 95% of sites where whipworm has been found. There are only two sites in the Medieval period where whipworm is found without roundworm. These are from a cesspit at Ile de la Cité, France and pelvic soil studied from Sai Island, Sudan.

Similarly there are only five sites where roundworm is found in the absence of whipworm these are the sewer from An Hof Wien in Austria, the pelvic soil from King Richard III, the latrines from Mértola in Portugal, the latrines from Córdoba in Spain, and the latrine from Gerasa in Jordan. As such I will not describe all the evidence for whipworm in the Medieval period as they have already been described in the evidence for roundworm.

2.3.7.2 Zoonotic Cestodes

In the Medieval period *Taenia* spp. eggs have been found in Belgium, Britain, France, Germany, Israel, and the Netherlands. In addition, eggs identified as *Taenia* or *Echinococcus* sp. have been found in Italy.

In Belgium, *Taenia* eggs were found alongside roundworm and whipworm in the latrine at Namur (da Rocha et al., 2006). In Britain, they were found in pit sediments from two sites in Medieval London (Rouffignac, 1985; Tibensky and Sidell, 2003). They were also found in a coprolite from York (Flammer et al., 2018). In France, they were found in cesspit samples from Ile de la Cité, Paris and a latrine at Marly-le-Roy (Bouchet et al., 1989; Bouchet et al., 1998). In Germany, aDNA analysis was used to identify beef tapeworm (*Taenia saginata*) in a latrine from the site of Lübeck. In Israel, *Taenia* sp. eggs were found in a latrine at Acre (Mitchell and Tepper, 2007) and coprolites from a Mamluk period latrine in Jerusalem (Yeh et al., 2015). In the Netherlands, eggs from *Taenia* sp. were found in a latrine at Kampen, and their presence was confirmed by aDNA analysis, though this did not yield a species-level identification (Søe et al., 2018).

Finally, in Italy eggs that were either *Echinococcus* sp. or *Taenia* sp. were found in a cesspit alongside roundworm and whipworm (Florenzano et al., 2012). In most cases, unless aDNA analysis was done, it was not possible to distinguish between pork and beef tapeworm as the eggs are identical.

Fish tapeworm has been found at nine Medieval period sites, three more than the Roman period. These sites are located in Belgium, Britain, the Czech Republic, France, Germany, Israel, Italy, and the Netherlands. In the Roman period fish tapeworm has only been found in Britain, France, and Israel.

In Belgium, *Diphyllobothrium* sp. eggs were found in the cesspit at Namur. In Britain, eggs identified as *Diphyllobothrium latum* came from pit sediments in Medieval London (Rouffignac, 1985). *Diphyllobothrium latum* was also identified in 13th–18th c. CE cesspits from Chrudim, Czech Republic (Bartošová et al., 2011), latrine samples from Montbéliard, France (Gonçalves et al., 2003), and both a latrine and cesspit from Acre in Israel (Mitchell et al., 2008; Mitchell et al., 2011). *Diphyllobothrium* sp. eggs were also identified in a coprolite from Mamluk period Jerusalem (Yeh et al., 2015) and a cesspit in Italy (Florenzano et al., 2012).

However, the only conclusive evidence for a species-level diagnosis of fish tapeworm comes from latrine samples from Lübeck in Germany that were analysed using aDNA (Flammer et al., 2018). *D. latum* has typically been considered the most, if not only, relevant *Diphyllobothrium* species in Europe (Garcia, 2016). Due to difficulties in distinguishing the eggs based on morphology, most previous species-level diagnoses were based on the known geographical distribution of the eggs, and eggs found in human contexts were often assumed to be *D. latum*. However, another species known to be present in Europe is *D. dendriticum* which cannot be easily distinguished from *D. latum* based on morphology of the eggs though *D. dendriticum* eggs tend to be slightly smaller (Leštinová et al., 2016). Other work has shown that subtle differences in surface texture and size may be used to differentiate some species of *Diphyllobothrium* (Le Bailly et al., 2005). Though the latrine samples were studied using aDNA, Søe and colleagues (2018) were unable to determine the species of *Diphyllobothrium* present in samples from Kampen.

2.3.7.3 Zoonotic Trematodes

In the Medieval period the lancet liver fluke (*Dicrocoelium* sp.) has only been found at 12 sites, the majority of which are in France. However, it has also been found in Britain and Italy.

In Britain, the lancet liver fluke has been found in two samples from Winchester, including cesspits and occupation layer sediments (Taylor, 1955; Pike and Biddle, 1966).

In France, there is a relatively large collection of literature identifying the lancet liver fluke in the Medieval period. Eggs were recovered from occupation layer sediments from Collège de France (Le Bailly and Bouchet, 2010). Then in occupation layer sediments from Mikelauen-Zilo (Le Bailly and Bouchet, 2010), coprolites from Charavine, and latrine sediments from the Grand Louvre, Paris (Bouchet, 1995). From the 12th–16th c. CE, evidence comes from cesspits at Ile de la Cité, Paris, samples taken from tombs at Villiers-le-Bel, and

latrine samples from Epinal, Rigny, Montbéliard, and Rue Cardinal Lemoine, Paris (Bouchet et al., 1989; Bouchet et al., 1995b; Le Bailly and Bouchet, 2010). Though there are many species of *Dicrocoelium,* which can be difficult to differentiate on microscopy, the only species which is known to be present in Europe is *Dicrocoelium dendriticum*. As Le Bailly and Bouchet (2010) pointed out in their review of dicrocoeliosis, the geographic distributions of the different species are such that most eggs of *Dicrocoelium* found in past populations in Europe should be *D. dendriticum*.

The only other evidence for lancet liver fluke comes from refuse pits in Piazza Garibaldi, Parma, Italy (Florenzano et al., 2012). These refuse pits contained different helminth eggs compared to the cesspit from the site which contained eggs from *Taenia* or *Echinococcus* sp., roundworm, whipworm. It is possible that the *Dicrocoelium* sp. eggs in this pit were from infected animals or animal refuse thrown in the pit. As *Dicrocoelium* is a common parasite of sheep, deer, and other animals this seems more likely than true human infection which is acquired from ingestion of ants or false parasitism if liver of an infected animal is eaten.

Fasciola liver fluke has been identified in samples from six different sites in Medieval period Europe, Middle East, and North Africa. These sites are located in Belgium, Britain, the Czech Republic, and France. There is no evidence for *Fasciola* liver fluke in Medieval Italy though it was found there during the Roman period; in fact the only evidence for *Fasciola* sp. in Italy is from the cores taken from Portus whose dates span the Roman period (Dufour, 2015).

The evidence for *Fasciola* liver fluke in the Medieval period comes from latrine sediments taken from the site of Namur, Belgium (da Rocha et al., 2006), and cesspit sediment from Chrudim, Czech Republic (Bartošová et al., 2011). In Britain, *Fasciola* liver fluke was found in pit sediments from London (Rouffignac, 1985), and cesspit sediments from Medieval period Leicester (Boyer, 1999). In France, it was found in cesspit sediments from Ile de la Cité, Paris and latrine sediments from Marly-le-Roy (Bouchet et al., 1989; Bouchet et al., 1998).

*2.3.7.4 Protozoa (*Entamoeba histolytica *and* Giardia duodenalis*)*

Entamoeba histolytica and *Giardia duodenalis,* two of the most common protozoa causing severe diarrhoea, have been found in the Medieval period. The earliest evidence for *Giardia duodenalis* in areas that were once part of the Roman Empire comes from Roman-period Turkey at the site of Sagalassos (Williams et al., 2017). In the Medieval period, we have more

extensive evidence for *Giardia duodenalis* than in any other time period, from ELISA analyses. Positive results came from five sites in the Czech Republic, France, Israel, and Switzerland (Gonçalves et al., 2002; Le Bailly, 2005; Le Bailly et al., 2008; Mitchell et al., 2008; Bartošová et al., 2011). Similarly, *Entamoeba histolytica* has been found at all of these sites as well except Chrudim in the Czech Republic.

In addition, *Entamoeba histolytica* has been found in the absence of *Giardia duodenalis* in Brouage and Villiers-le-Bel in France and Namur in Belgium. Positive results have come from a mix of sample types. Most are from cesspits or latrines, but both protozoa were found in occupation layer sediment from Pineuilh, France; *Entamoeba histolytica* was found in sediment from a tomb at Villiers-le-Bel, France; and both protozoa were found in pelvic soil from Chevenez in Switzerland.

*2.3.7.5 Other Helminths (*Echinococcus *sp.,* Hymenolepis *sp., Leishmaniasis, Pinworm*, *Schistosomiasis*, Toxocara *sp.)*

Evidence for *Echinococcus granulosus,* or hydatid disease, has been found at one Medieval period site in Switzerland (Baud and Kramar, 1991). A calcified hydatid cyst was found with the skeletal remains of a child.

The dwarf tapeworm, *Hymenolepis* sp.*,* has been found at Chrudim, Czech Republic (Bartošová et al., 2011) as well as Sedeinga and Sai Island, Sudan (Harter, 2003). In Sudan these eggs were found in pelvic soil samples whereas evidence from the Czech Republic is from a cesspit. There is no previous evidence for *Hymenolepis* sp. in the Roman period or earlier periods in Europe, the Middle East, or North Africa.

Pinworm has been found at three sites in the Medieval period. The first is from cesspit samples from the site of Chrudim in the Czech Republic, the second is from pelvic soil from burials at Sedeinga in the Sudan, and the third from a latrine at Kampen in the Netherlands (Harter, 2003; Bartošová et al., 2011; Søe et al., 2018). In the Roman period the only evidence for pinworm came from Egypt and Israel.

Toxocara canis, commonly called the dog roundworm, was identified in 13th–18th c. CE cesspits from Chrudim, Czech Republic (Bartošová et al., 2011). Eggs from *Toxocara* sp. were also found in two latrines from Kampen, Netherlands though DNA could be not be found in the samples. *Toxocara canis* can infect humans if embryonated eggs are ingested causing larva migrans, a condition where the worm migrates through the tissues causing irritation. However, it is more likely that the eggs found were from animal faeces deposited in

the latrines or were a result of false parasitism where the eggs were ingested accidentally and passed unchanged through the intestinal tract.

Evidence for schistosomiasis in the Medieval period comes from France and Sudan. *S. haematobium* and *S. mansoni* have been found at Montbéliard, France (Bouchet and Paicheler, 1995; Bouchet et al., 2002). The authors suggested that the presence of schistosome eggs in France may have been the result of movement of African slaves into France, as these parasites are typically found in Africa. *S. mansoni* has also been found in mummies from Sudan, at the sites of Kulubnarti and Wadi Halfa (Miller et al., 1992; Hibbs et al., 2011). Eggs identified to the genus *Schistosoma* but of an unknown species were found in pelvic soil from burials at Sedeinga, Sudan (Harter, 2003).

In addition to these helminths, aDNA analysis confirmed the presence of the protozoa *Leishmania donovani,* which commonly causes visceral leishmaniasis, in mummies from Kulubnarti, Sudan dated to 550–1500 CE (Zink et al., 2006).

2.4 Integrating the Evidence

As has been seen, there have been quite a few studies undertaken to identify parasites in Neolithic Europe and the Middle East which gives us good evidence to compare to parasites found in the Roman period. However, relatively few studies have been performed in Iron Age and Bronze Age Europe. By far, the Medieval period has the most data currently which should allow for a better understanding of how parasitic infection may have changed after the Roman period. We also have very little evidence for parasites in the Middle East and North Africa compared to Europe from all time periods. Based on climate differences in these areas, and the evidence for parasites that we do have, there are a variety of species endemic in these regions that are not seen farther north in Europe. Though there appears to be quite a bit of evidence for parasites in the Roman period, when we start to look at the distribution of any one species there is not enough information from all countries to make meaningful conclusions and, therefore additional studies on samples from all countries and especially understudied regions are required for more meaningful conclusions.

Currently, there is very little evidence for parasitic infection at the individual level, as most studies in all of these time periods have focused on latrines, cesspits, or occupation layer sediments. Finding parasite eggs in latrines, cesspits, and occupation layer sediments can give us a good indication of taxa present at a population level but limits our understanding of how they were distributed in the community, for example in different age groups. Studying pelvic

soil samples will allow us to further understand how parasite infections were distributed amongst individuals within a population.

When we look at species diversity through time, the published data to date suggest that species diversity was higher in Neolithic sites, with some sites having evidence for as many as nine different species. Based on what is known about the way of life in a Neolithic settlement, where population size was much smaller and the subsistence relied on farming or herding, we may expect to see more zoonotic parasites acquired from individuals living in close proximity to animals and still relying on wild animals as a food source. With larger population sizes and more densely populated cities in the Roman period, it appears that we are beginning to see an increase in species associated with poor sanitation or faecal contamination of food and water. Though the Romans had latrines, sewers, bathhouses, and some form of organized waste disposal it appears that this was not enough to prevent the spread of parasitic infection. Further evidence from a wider range of sites in the Roman Empire will allow us to look for patterns of infection in different areas of the empire and gain more information about how the prevalence of individual parasite species changed over this time period. We have a large body of evidence from the Medieval period to use as a comparison.

3 Methods

Two main methods were used to analyse all samples for preserved parasite eggs. The first is microscopic analysis and the second is enzyme-linked immunosorbent assay (ELISA). Microscopic analysis is used to look for eggs of intestinal helminths and ELISA is used to look for smaller protozoal parasites that are typically found in their inactive cyst form outside their host. Before either type of analysis can be performed the samples need to be prepared using rehydration and disaggregation and microsieving to concentrate any eggs or cysts present in the sediment. For an overview of the methods used see Figure 21.

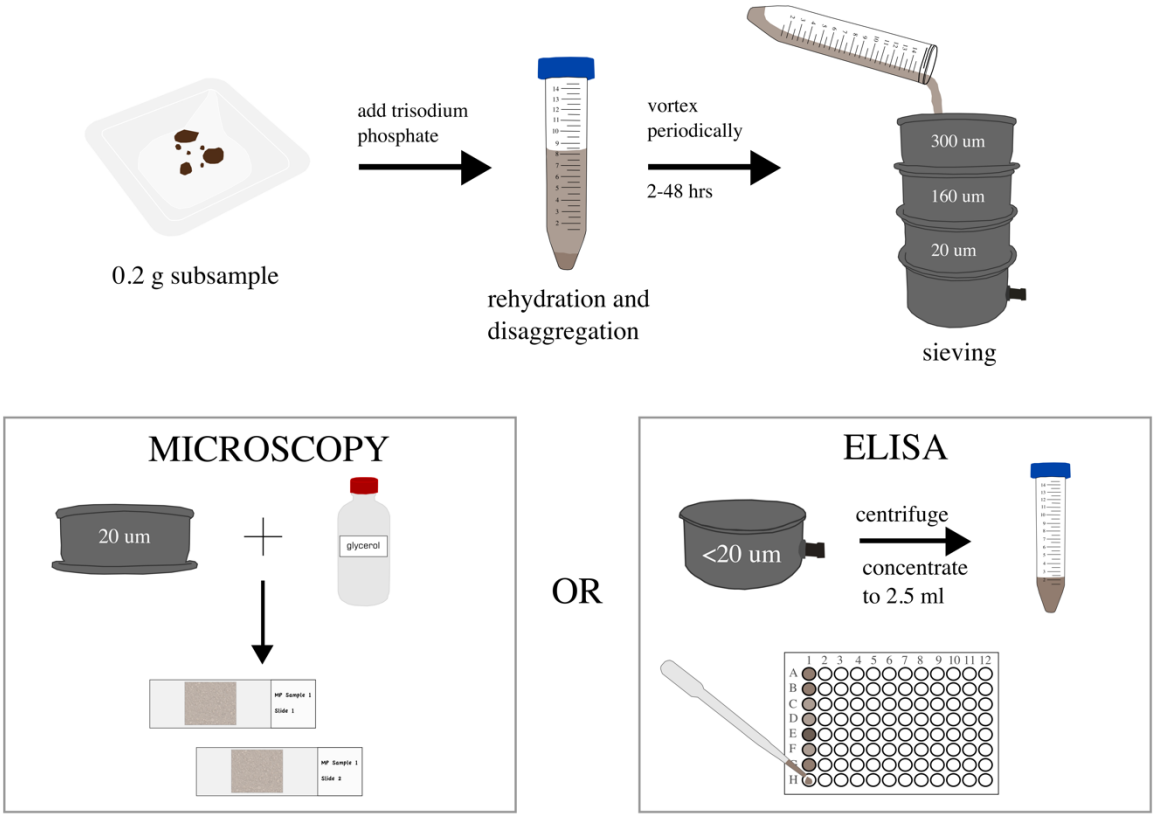

Figure 21: Overview of methods used with sample preparation depicted on the top and microscopy or ELISA on the bottom.

3.1 Sample Preparation

Rehydration and disaggregation was used to separate out the particles of interest. Disaggregation of the sample ensures that any parasite eggs that may be trapped in larger pieces of sediment are released into the liquid suspension. For each sample, a 0.2 g subsample was taken. This subsample was then disaggregated with 0.5% trisodium phosphate until all material was suspended in the liquid and soluble material was dissolved. The 0.2 g subsample was placed in a 15 mL test tube and ~8 mL of 0.5% trisodium phosphate was added. The sample was left in trisodium phosphate for a minimum of 2 hours or until all material was

disaggregated, with sand and other insoluble particulates floating freely in the liquid suspension. The samples were periodically shaken or mixed with a vortex every 30 minutes to ensure that all material was broken up.

Trisodium phosphate was used for the disaggregation process because it is well supported in the literature as an effective solvent to rehydrate and disaggregate various archaeological samples for parasite egg analysis without damaging parasite eggs (Callen and Cameron, 1960; Reinhard et al., 1986; Bouchet et al., 2001a; Bouchet et al., 2003; Fugassa et al., 2006). There is evidence that distilled water is equally as effective for disaggregation of latrine soil samples (Anastasiou and Mitchell, 2013b). In their study, Anastasiou and Mitchell did not find any substantial loss of taxa identified when they used distilled water for disaggregation. However, it is possible that a dilute salt solution like trisodium phosphate may still be better for rehydrating certain types of archaeological material such as dried coprolites, which were not tested in this study. The use of 0.5% trisodium phosphate for rehydration of palaeoparasitology samples was established by Callen and Cameron (1960) to allow them to identify parasites in coprolites from Peru. It has been shown to be effective for all sample types, and is still the current standard in the field. The use of trisodium phosphate was used for disaggregation of the samples studied in this dissertation as it has been shown to be equally effective as water and it is more widely accepted in the field.

For samples that do not disaggregate with trisodium phosphate (e.g. heavily mineralized material), addition of acids and bases can aid in visualization of parasite eggs by releasing these eggs from concretions. Dufour and Le Bailly (2013) evaluated the use of different acids and bases in preparing archaeological samples for paleoparasitological analysis. Acids, such as dilute hydrochloric acid, can be added to sediments to break down carbonates (Warnock and Reinhard, 1992). In samples that are heavily mineralized like concretions the addition of the acid can be helpful to release parasite eggs from this solid mass of material. Similarly, other acids and bases like sodium hydroxide and hydrofluoric acid can be added to break down organic and plant material if this material is unusually dense and obstructs parasite eggs from view (Dufour and Le Bailly, 2013). It should be noted that the addition of strong acids or bases has been shown to decrease the number of species of parasites identified so should only be used when analysis cannot proceed effectively without them (Dufour and Le Bailly, 2013). The only samples studied here that required the use of additional chemicals for disaggregation were the concretions from the private latrine at Ephesus and the coprolite from Viminacium. These samples would not disaggregate with trisodium phosphate so dilute hydrochloric acid was added. It is expected that dilute acids

have less of an effect on parasite eggs than the strong acids tested by Dufour and Le Bailly (2013).

In the two cases where it was needed, dilute hydrochloric acid (~10%) was added dropwise to the subsample until the reaction started, evident by bubbling as CO_2 gas was released from the breakdown of carbonates. HCl was continuously added to keep the reaction going until the material had completely broken up. The sample was centrifuged at 4,000 rpm for 5 minutes, the supernatant removed and the 15 mL tube filled with distilled water, mixed and recentrifuged. The supernatant from the addition of distilled water was removed and the tube refilled with distilled water for a total of five washes to ensure that the sample was brought back to a neutral pH.

After samples were fully disaggregated they were then passed through a set of three microsieves. The purpose of the microsieves is to separate out the particles that are similar in size to parasite eggs. This approach is also used by labs in France (Bouchet et al., 2001a; Dufour and Le Bailly, 2013). In the United States, labs use a single mesh measuring 300 µm and sedimentation by centrifugation to concentrate parasite eggs (Reinhard and Urban, 2003; Camacho et al., 2018). For processing of the samples studied here, a stack of three sieves with a catchment container at the bottom was used. The sieves have mesh sizes of 300 µm, 160 µm, followed by 20 µm. The disaggregated sample was poured onto the top (300 µm) sieve and the material was thoroughly washed through with distilled water. The material that was deposited on the 160 µm sieve was also washed through with distilled water, and the material that remained on the top of the 20 µm sieve was collected and put into a 15 mL test tube. Any parasite eggs present in the samples should fall between the 20 µm and the 160 µm sieves, as the typical size range of human intestinal helminth eggs in this part of the world is 20-140 µm (Garcia, 2016). Protozoal cysts are often smaller than 20 µm but these can be detected using ELISA, as will be discussed later. The collected material was then centrifuged at 4,000 rpm for 5 minutes. Centrifuging results in the remaining sample particles, and any parasite eggs present, being separated from solution to the bottom of the test tube and then the clear supernatant can be removed from on top of this pellet. The remaining material was then processed to visualize any parasite eggs using microscopy.

To ensure no contamination between samples, sieves were cleaned between each use. The sieves were placed in an ultrasonic bath with DECON 90 (a detergent) for 30 minutes, then thoroughly rinsed and dried before being used for the next sample. All other materials used were consumable, including plastic weigh boats, centrifuge tubes, and single use plastic pipettes.

3.2 Microscopy

Once the archaeological sample was disaggregated and centrifuged, it was mixed with glycerol and placed on microscope slides to be visualized. Parasite eggs were then identified based on morphological characteristics (Table 10). Parasitology reference manuals and previously published palaeoparasitological results were used as an aid for egg identification (World Health Organization, 1994; Ash and Orihel, 2015; Garcia, 2016). The size of helminth eggs is relatively constant and usually only varies from 5 to 20 µm between eggs of the same species (Garcia, 2016). The key features used to diagnose parasite eggs are size, shape, symmetry, uniform egg shell thickness, colour, and any unique features (e.g. polar plugs or opercula). Other material that is often present in archaeological samples that can be difficult to distinguish from parasite eggs are fungal spores, pollen, and plant material. However, in most cases it should be possible to distinguish parasite eggs from other material if close attention is paid to the size and morphology of the eggs.

In some cases presence or absence of certain morphological features and shape of eggs can hint at the preservation of the eggs. For example numerous eggs with preserved polar plugs, in the case of *Trichuris* sp. and *Capillaria* sp. eggs, is often found in sites with good preservation (Morrow et al., 2014; Rácz et al., 2015). Similarly presence of mamillated coats on *Ascaris* sp. eggs is often found in areas with good preservation though this is not always the case (Morrow et al., 2016); and modern eggs have been shown to lose their mamillated coats after exposure to different chemicals (Oh et al, 2016).

Table 10: Characteristics used to identify common helminth eggs found in archaeological sediments in Europe.

Parasite	Egg Dimensions	Characteristic morphological features
Ascaris lumbricoides (roundworm)	Fertile Length: 55–75 µm Width: 35–50 µm Infertile Length: 85–95 µm Width: 43–47 µm	Round to oval shape Thick shell Smooth surface or covered in mamillations (typically yellow to brown in colour)
Capillaria sp.	Length: 36–67 µm Width: 30–45 µm (size ranges are more narrow for specific species)	Thick shell Two opposing polar plugs with shallow prominences Reticulated or striated shell surface
Dicrocoelium dendriticum (lancet liver fluke)	Length: 38–45 µm Width: 22–30 µm	Thick brown shell Operculum
Diphyllobothrium sp. (fish tapeworm)	Length: 58–75 µm Width: 40–50 µm	Oval shape Moderately thick shill Operculum, often indistinct (continuous with outer shell) Knob at abopercular end
Taenia sp. (beef or pork tapeworm)	Diameter: 31–45 µm	Spherical shape Thick yellow/brown shell with radial striations
Trichuris trichiura (whipworm)	Length: 50–55 µm Width: 22–24 µm	Thick yellow/brown shell Lemon-shaped Two opposing polar plugs with clear colour Smooth surface

Note: dimensions are from Ash and Orihel (2015); morphological characteristics from Ash and Orihel (2015) and Garcia (2016).

A total of five slides from each subsample were viewed and if no parasite eggs were found no more slides were analysed. If any eggs were found then the entire 0.2 g subsample was analysed in order to calculate the concentration of eggs (see below). While it is possible that some parasite eggs were missed by not viewing larger subsamples, it has been reported that in 75% of samples the maximum number of taxa was identified within the first six slides, and any taxa identified in the following slides are represented by very few eggs (Dufour, 2015). Five slides were analysed in order to balance the ability to identify all taxa present in a sample and analysing larger numbers of samples from multiple individuals or areas of a site. In this research it was also observed that analysing additional slides after the first five did not result in identification of new taxa in most cases.

Microscopy is the most common method used for detection of parasites in palaeoparasitology. This is likely due to a number of factors; mainly that it is relatively inexpensive and though slightly more time consuming than some other methods it can be used to diagnose most intestinal parasites. Microscopy has remained a core method in palaeoparasitology since the advent of the field. In fact, it is also an important diagnostic tool in clinical parasitology. Though preparation of modern human faecal samples for microscopic ova and parasite analysis differs from preparation of archaeological samples, the principles of identifying the parasites under the microscope is very similar. In clinical medicine adult worms, and helminths at various stages of development can be seen (e.g. larvae and eggs), however in archaeological samples eggs are typically the only developmental stage that are preserved well enough to be visualized. One exception to this is in mummified remains where adult worms and larvae may be preserved due to the same processes that allow the human tissues to resist decomposition.

Even though microscopy is the main method used clinically and archaeologically to detect parasitic infection there are some major limitations to microscopic analysis. First, it can be difficult to make a species-level diagnosis in all cases. Some animal species have eggs that are indistinguishable from the human species. This is the case for roundworm infecting pigs (*Ascaris suum*) and roundworm infecting humans (*Ascaris lumbricoides*) (Betson et al., 2014). There are also cases where different species of human parasites cannot be differentiated. For example, beef and pork tapeworm, *Taenia saginata* and *Taenia solium*, respectively. The eggs of both of these species are nearly identical and the only way to differentiate the two would be to undertake ancient DNA (aDNA) analysis. For other intestinal parasites such as fish tapeworm (*Diphyllobothrium* spp.) and *Capillaria* spp., differentiating the species based on morphology of the eggs alone can be quite difficult but is possible. For example, there are 16 different species of *Diphyllobothrium* that can infect humans (Leštinová et al., 2016). Four of these, including *D. latum*, *D. pacificum*, *D. dendriticum*, and *D. nihonkaiense*, are common causes of fish tapeworm infection in humans. It has been argued that it is not possible to differentiate these different species using microscopic analysis of the eggs (Holiday et al., 2003). In the past, in both modern parasitology and palaeoparasitology, eggs of fish tapeworm were often identified broadly as *Diphyllobothrium* sp. or simply as the most common species known to infect humans, *Diphyllobothrium latum* (Kuchta et al., 2013). However, more recently there have been a number of studies undertaking analysis of a larger number of eggs from different species and different hosts that have argued that size along with differences in depth and density of pores

on the eggshell can in fact be used to differentiate between species of *Diphyllobothrium* (Le Bailly and Bouchet, 2013; Leštinová et al., 2016).

Furthermore, in some cases, taking into consideration the different geographic distributions of species of the same genus can inform which species is most likely to be present in the samples being studied. This of course assumes that the infected individual had not migrated from an area where different species were endemic. For example, Le Bailly and Bouchet (2010) have argued that the majority of *Dicrocoelium* eggs in Europe are likely from *Dicrocoelium dendriticum* (the lancet liver fluke) because this is the only species of *Dicrocoelium* found in Europe today. This type of assumption can be helpful, though we have to remember that the geographical distribution of species in the past could have been very different from what we find today. In fact, this is something we are trying to understand by studying parasitic infections in past populations.

3.3 ELISA

ELISA is another common method used in palaeoparasitology. The first study to use ELISA to detect protozoan parasites in archaeological sediment samples was in 1999. Allison and colleagues used a commercially available ELISA kit to detect *Giardia duodenalis* and *Cryptosporidium* (Allison et al., 1999). Since this time, ELISA has become a well-established method to be used alongside microscopy in order to detect intestinal protozoa. Gonçalves and colleagues (2004) tested samples from 15 different sites for *Entamoeba histolytica* and Le Bailly and Bouchet (2015) have recently published a review of data for *Entamoeba histolytica* in the past showing that almost all information for this organism has come from studies using ELISA. Of the three protozoa which can cause severe diarrhoea in humans, we have the most evidence for *Entamoeba histolytica*, but our knowledge of the distribution of *Giardia duodenalis* in the past has increased greatly with the use of ELISA (Gonçalves et al., 2002; Le Bailly et al., 2008; Mitchell et al., 2008). Evidence for *Cryptosporidium* in past populations is still sparse, with only a few positive results in South America (Allison et al., 1999; Ortega and Bonavia, 2003).

When undertaking lab analysis of archaeological samples using ELISA kits, the samples were prepared using the same disaggregation process as was used for microscopy. However, 1 g subsamples were used and when the sample was sieved the material from the catchment container underneath the 20 µm sieve was collected. This is because the cysts of the protozoa being studied are typically smaller than 20 µm (Garcia, 2016). The material collected from the catchment container was placed in a 15 mL test tube and centrifuged at

4,000 rpm for five minutes. The clear supernatant is then removed, leaving 2.5 mL of sample in the tube to be used in the three ELISA kits, one for each organism of interest.

The ELISA kits used for the analysis were commercial TechLab kits, which are validated for use in a clinical setting. For detection of *Entamoeba histolytica*, the E. HISTOLYTICA II kit was used. For *Giardia duodenalis,* the GIARDIA II kit was used and for *Cryptosporidium,* the CRYPTOSPORIDIUM II kit. The tests were undertaken following the procedures laid out in the manuals provided with the kits. Each kit contains an ELISA plate with 96 test wells. Each well has an organism-specific antibody immobilized to the plate. For the *Entamoeba histolytica* kit this is an antibody specific to the galactose adhesin found on *E. histolytica* cysts. For the *Giardia duodenalis* kit the antibody is specific for a cell-surface antigen, and the *Cryptosporidium* kit uses an antibody specific for an oocyst antigen. When the sample is added to the well, if the organism or even just the antigen we are looking for is present it will bind to the antibody on the plate and be stuck to the plate. A second antibody linked to an enzyme is then added to each well and will bind to the antibody-antigen complex already present in any wells containing the organism. The addition of a substrate will interact with any enzyme-antibody-antigen complexes formed resulting in a colour change in these wells. The colour change is a visible yellow, however for increased accuracy an ELISA plate reader was used to determine the exact absorbance value of each well. After addition of the stop solution, which produces the yellow colour change, the plates were incubated for 2 minutes and absorbance values were read within five minutes after this. A BioTek Synergy HT ELISA plate reader was used at 450 nm to acquire the absorbance values for each well, which were then compared to the TechLab published values to determine which wells were positive. For *Giardia* and *Cryptosporidium,* a positive read is an absorbance value ≥ 0.150. For *Entamoeba,* a positive read is an absorbance value that is 0.050 or higher after the value from the negative control well has been subtracted. For each ELISA plate, an entire column (8 wells) was dedicated to each sample. The same sample was placed in each of 8 test wells, acting as a technical repeat. Each plate had one positive and one negative control well to validate the plates. The positive controls are provided with each kit and are an antigen from the organism of interest.

The TechLab kits have high sensitivity and specificity for the parasites they test for with no cross reactivity with other organisms. For the *E. histolytica* kit the sensitivity is 96.9% and specificity is 100% and it has no cross-reactivity with *E. dispar*, for *Giardia duodenalis* the sensitivity and specificity are 100%, and for *Cryptosporidium* the sensitivity is 97.7% and specificity is 100%. However, it is important to note that the kits were designed

for use in a modern clinical setting where fresh or frozen faecal samples would be tested for protozoan organisms. The sensitivity and specificity of these tests is likely different when they are applied to archaeological samples because the antigens (proteins) being tested for degrade over time resulting in changes to their tertiary structure and reducing their binding capacity (Brown and Brown, 2011) potentially resulting in higher false negatives. Though much less likely due to the specificity of the antibodies, we cannot rule out that changes to protein structure could result in false positives. For this reason, each test is rerun on a different day using a new subsample of material, this second test with a new subsample represents the biological repeat. A sample is only considered to have a positive result if positive wells are present on both tests.

The benefits of using ELISA in combination with microscopy is that we can detect protozoan parasites that are typically found in cyst form rather than egg form in archaeological samples, and as such do not preserve as well to be directly visualized. While there have been reports of protozoan cysts being seen under the microscope it is quite rare. This is because the cyst walls are much thinner than eggshells and are much more susceptible to degradation (Mitchell, 2015a). By using ELISA to detect only one specific antigen we can detect any complete cysts as well as any poorly preserved cysts as long as the antigen is still present in a form that can bind to the antibody and that they are present in high enough concentrations to trigger a positive result.

3.4 Egg Quantification Techniques

In order to quantify the number of eggs present in each subsample studied the entire 0.2 g sample was analysed and the total number of eggs found for each taxa was counted, which is equivalent to the number of eggs in 0.2 g of sediment. This number was multiplied by five to arrive at the number of eggs per gram (epg).

$$epg = eggs\ counted \times 5$$

There are other methods for quantifying eggs including the use of *Lycopodium* spore tablets (Warnock and Reinhard, 1992; Fugassa et al., 2006; Jiménez et al., 2012). These tablets contain known amounts of *Lycopodium* spores and the dissolved tablet is added at the rehydration and disaggregation phase of sample preparation. As parasite eggs are identified, spores are also counted and an approximate concentration of eggs can then be calculated based on the number of spores counted.

$$epg = \left(\frac{eggs\ counted}{spores\ counted}\right) \times \left(\frac{known\ quantity\ of\ spores}{subsample\ weight\ (g)}\right)$$

3.5 Skeletal Age and Sex Estimations

Age-at-death and sex estimations were available from collaborators for many of the pelvic soil sample sets. From Vagnari these estimations were done by myself and Dr. Tracy Prowse. I will describe our methods for these estimations, the methods used by other collaborators are similar though there may be slight variations. Age and sex estimations were collected in the hopes of understanding distributions of parasitic infections within the skeletal samples.

First, age estimations were made using both the dentition and skeleton. For juveniles, tooth eruption and development for both deciduous and permanent dentition was recorded and compared to reference ranges (Gustafson and Koch, 1974; Buikstra and Ubelaker, 1994). In addition, fusion of epiphyses of various bones occurs at relatively standard ages and these were also recorded (McKern and Stewart, 1957; Buikstra and Ubelaker, 1994). Finally, in some infants who had no teeth present, the length of long bones was used for a, generally less specific, estimation of age (Scheuer and Black, 2000). Tooth development has been found to be more consistent with chronological age than bone development (White and Folkens, 2005). It needs to be kept in mind that certain diseases such as parasitic infection can lead to stunting of growth meaning that an age estimation made from long bone lengths alone may underestimate the actual age of the individual. In any case, all age estimations in archaeological settings are given as a range usually of years not months, except in very young individuals.

In adult individuals where dentition is fully erupted and bones are fused, other bony traits were used for age estimation including the appearance of the auricular surface on the os coxa and the pubic symphysis (Buikstra and Ubelaker, 1994). In many cases these skeletal elements were not preserved or were unobservable and age could not be estimated beyond classifying the individual as an adult.

Sex estimations were undertaken for individuals who had gone through puberty, as this is when development of secondary sex characteristics results in better observed sex differentiation in the skeletal remains. Morphological features on both the cranium and pelvis were used. Features on the cranium including the supraorbital ridges, glabellar region, nuchal lines, mastoid process, and shape of the mandible were recorded (Buikstra and Ubelaker, 1994). On the pelvis, the shape of the greater sciatic notch and the three features used in the Phenice method (Phenice, 1969) including ventral arc, subpubic concavity, and the medial aspect of the ischiopubic ramus were observed. For juveniles (<18 years) no sex estimation was made and these individuals were listed as undetermined. Similarly, for skeletons that did not have good enough preservation for sex to be estimated, their sex is listed as undetermined.

In the case of adult individuals where the various morphological features were inconclusive, falling in the mid-range for male and female traits, these individuals were recorded as indeterminate.

4 Study Sites and Results

4.1 Materials Overview

The samples analysed for this dissertation were chosen to fill in some of the major gaps in our knowledge about intestinal parasites in the Roman Empire. As such, there are two main foci; 1) samples from the Mediterranean region, especially the eastern Mediterranean, and 2) pelvic soil samples that can give more concrete evidence for infection at an individual level in regions where we have good comparative data. In addition to samples from Roman sites, a number of samples from earlier and later time periods were studied to increase the amount of comparative data. The collection of samples was limited by availability of material from previous and ongoing excavations. Collaborations were established to study samples from each site, and the key individuals involved in collection of samples and discussion of context of the samples are listed in the Acknowledgments section and the materials section for each site.

First, the Roman period samples come from twelve different sites and are a mix of sample types (Table 11). In the understudied Mediterranean region I analysed samples from Cyprus, Italy, Serbia, and Turkey (Figure 22). In Northern Europe where we have a lot more palaeoparasitological work to use as comparative material I studied pelvic soil samples from two cemeteries in the United Kingdom and one in Austria as well as latrine sediments from Belgium. From some sites multiple latrines or drains were studied and some also had multiple layers from inside one latrine or drain. As such a total of 98 individuals or features were studied from Roman sites while a total of 112 samples were analysed, not including control samples.

Table 12 lists the comparative samples studied as part of this work. Though it was not possible to collect or analyse enough samples to even out geographic or temporal coverage, these samples were a start to filling in major gaps in our understanding of pre-Roman and post-Roman parasites in regions that at one point were part of the Roman Empire. For example, very few archaeological samples had been analysed for parasites from Turkey in any time period, prior to this dissertation, so samples were collected from other time periods to provide some comparative evidence. In fact, no other ancient parasite studies had been undertaken in Turkey except from the Roman site of Sagalassos which was completed by our lab during this doctoral research (Williams et al., 2017).

Table 11: Details of samples analysed from Roman sites.

Site	Country	Roman Province	Date	Sample Type	Number of Samples
West Smithfield, London	UK	*Britannia*	Roman	Pelvic soil	30
Winterborne, Dorset	UK	*Britannia*	Roman	Pelvic soil	5
Lauriacum	Austria	*Noricum*	3rd–4th c. CE	Pelvic soil	13
Arlon	Belgium	*Belgica*	250–280 CE	Latrine	1
Paphos	Cyprus	*Cyprus*	Hellenistic/ Roman	Pelvic Soil	6
Oplontis	Italy	*Italia*	79 CE	Pelvic soil	8
Vagnari Cemetery	Italy	*Italia*	2nd–4th c. CE	Pelvic soil	22
Vagnari Vicus	Italy	*Italia*	2nd–4th c. CE	Drains	4
Vacone	Italy	*Italia*	Roman	Latrine/drains	3 (10)
Viminacium	Serbia	*Moesia Superior*	2nd–3rd c. CE	Coprolites	1(4)
Ephesus	Turkey	*Asia*	3rd–6th c. CE	Latrines	2 (5)
Sardis	Turkey	*Asia*	6th–7th c. CE	Drains	3 (4)
Total					**98 (112)**

Notes: Number of samples listed refers to either number of individuals sampled for pelvic soil or number of features sampled if they are from latrines and drains. The numbers in brackets are the total number of samples analysed as some latrines and drains had multiple layers and the coprolite from Viminacium had multiple subsamples taken from it due to its large size.

Table 12: Comparative samples analysed from other time periods.

Site	Country	Date	Sample Type	Number of Samples
Must Farm	UK	9th c. BCE (Late Bronze Age)	Occupation sediments	14
Must Farm	UK	9th c. BCE (Late Bronze Age)	Coprolites	15
Hatherdene Close	UK	5th–6th c. CE (Anglo-Saxon)	Pelvic soil	92
Çatalhöyük	Turkey	7100–6300 BCE (Neolithic)	Pelvic soil	7
Boncuklu	Turkey	8300–7800 BCE (Neolithic)	Pelvic soil	9
Griffin Warrior, Pylos	Greece	1450–1400 BCE (Bronze Age)	Pelvic soil	1
Vacone	Italy	608–770 CE (Lombard)	Pelvic soil	6
Total				**138**

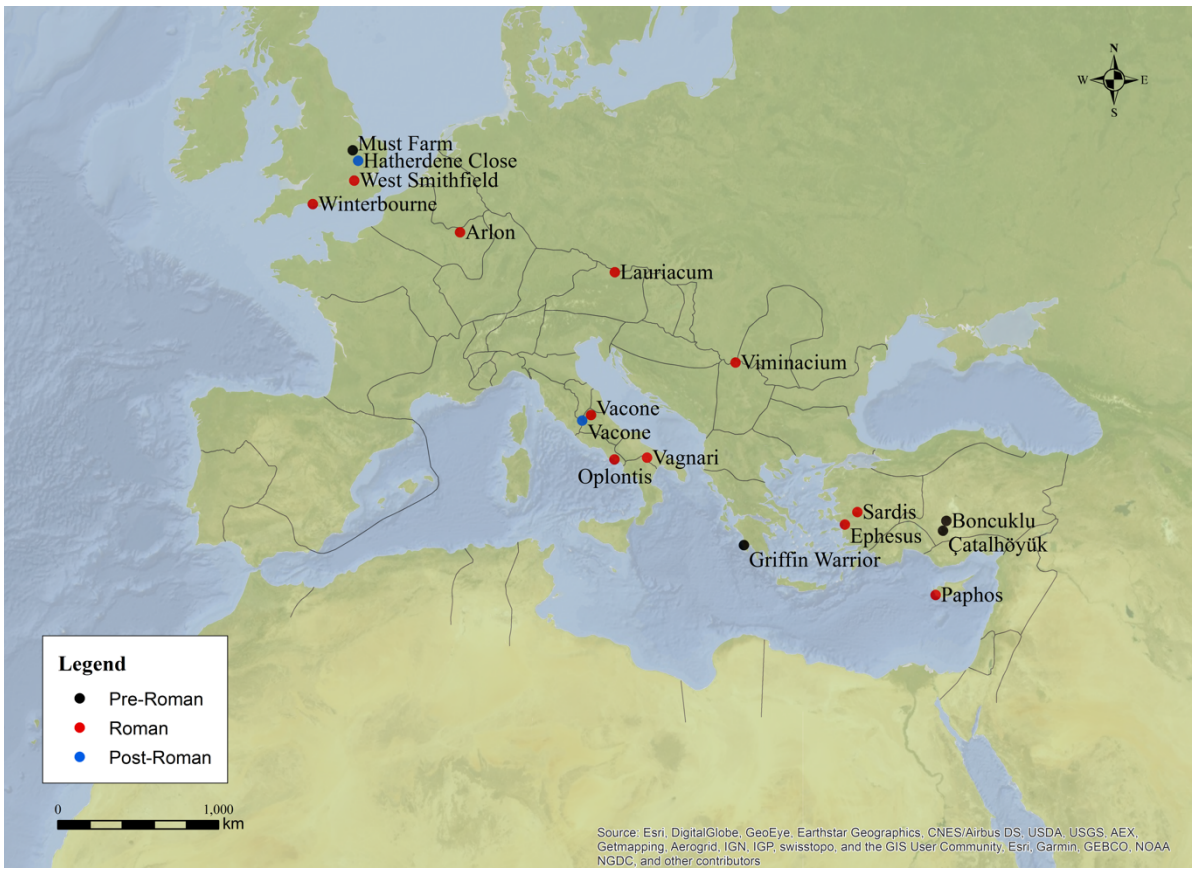

Figure 22: Location of sites studied. Black dots are pre-Roman sites, red dots are Roman sites, and blue dots are post-Roman sites. Roman provincial boundaries (117 CE) are from the Digital Atlas of Roman and Medieval Civilizations, Harvard University.

4.2 Pre-Roman Sites

4.2.1 The Must Farm Settlement

4.2.1.1 Materials

The parasitological analysis of samples from the Must Farm settlement has been published (Ledger et al., 2019a, see Appendix A.1). As such, material in this dissertation is adapted from that publication and a chapter written for the site monograph.

The Late Bronze Age pile-dwelling settlement at Must Farm was located in eastern England in an area known as the Flag Fen basin, which was part of a large wetland covering 46,000 km^2. The Flag Fen basin was formed with rising sea levels after the last glacial maximum, and was later drained in the 17th century CE. This wetland area has a number of other important prehistoric archaeological sites including Flag Fen and Fengate (Pryor, 2001). During the Bronze Age this wetland area contained numerous bodies of freshwater and brackish water interspersed by pockets of dry land, with periodic fluctuations in water levels (French, 2003: 108). The settlement at Must Farm consisted of a series of five stilted timber structures surrounded by a palisade, all built over this freshwater. Analysis of insect and plant

remains at the site shows that the water beneath the settlement flowed at an extremely slow rate and contained dense aquatic plants.

Excavations of the site were undertaken in two seasons; a small exploratory excavation in 2006 (Gibson et al., 2010; Robinson et al., 2015) and a larger excavation of the rest of the site from 2015–2016. These excavations revealed an exceptionally well-preserved Late Bronze Age settlement and associated artefacts including wood, textiles, ceramics, and metalwork. Radiocarbon dates place the period of occupation of the settlement during the 9th century BCE, which is the terminal Bronze Age in southern Britain (Knight et al., 2019). Dendrochronological evidence suggests that the settlement was constructed and inhabited for a very short period, around one year, before a large fire tore through the settlement causing the structures to collapse into the channel below where they were subsequently covered in alluvial silts. This waterlogged and anaerobic environment created ideal conditions for the preservation of organic and inorganic material from the Late Bronze Age settlement.

Occupation layer sediment and coprolites were collected for parasite analysis during the 2015–2016 excavations of the site. The sampling strategies were adapted from those that have been found effective at other prehistoric stilted settlements in Europe (Dommelier et al., 1998; Maicher et al., 2017). Sediment samples were collected by the site excavators under the direction of Rachel Ballantyne and Mark Knight. Samples were collected from three identified layers or contexts; the construction/occupation layer, the conflagration ash, and the post-conflagration river silts. Structures 1–4 were sampled, but Structure 5 was not sampled due to its prior excavation for evaluation of the site in 2006. Two locations from each structure were sampled; one from underneath the centre of the structure, and one from the outer edge of the structure. The samples from the outer edges of the structures were taken in locations where other refuse was found. These refuse locations were chosen because it was thought that they were the most likely areas where humans would dump excrement. Single samples were also taken from upstream of the entire settlement and downstream of the entire settlement (Figure 23). All samples were collected by the excavators of the site. The two construction/occupation layer sediment samples from Structure 3 were studied by an undergraduate student, Madison Fairey. The two construction/occupation layer samples from Structures 2 and 4 were studied by an MPhil student, Elisabeth Grimshaw. I analysed all other layers and samples from other locations, eight samples in total. I also analysed all coprolites.

Waterlogged coprolites (preserved pieces of faeces) were also collected from around the structures; a total of fifteen were used for analysis (Figure 23). Faecal biomarker analyses were conducted on the coprolites to determine their source, work done by Ian D. Bull and

Helen Whelton from the University of Bristol. This approach uses gas chromatography-mass spectrometry (GC-MS) to characterize 5β-stanols and bile acids in the coprolites. These lipids are present in faeces as a result of foods eaten and from biological processes. The ratios of 5β-stanols are used to distinguish omnivore (e.g. pig, human, or canine) from ruminant coprolites (Evershed and Bethell, 1996). 5β-stanol profiles dominated by coprostanols are indicative of human or pig origin while those dominated by cholesterols are indicative of canine origin (Bull et al., 2002). Bile acid profiles can then be used to distinguish pig from human faeces (Simpson et al., 1999). Eleven of the fifteen coprolites yielded lipid profiles adequate for source identification. Four were of human origin (SF3082, SF3127, SF3631, and SF3642) and seven were of canine origin (SF3175, SF3177, SF3178, SF3492, SF3677, SF3955, and SF4029) while the remaining four had inconclusive profiles due to high levels of compounds from the surrounding environment (SF3080, SF3176, SF3365, SF4034). However, the size and morphology of these coprolites is consistent with the coprolites of identified origin, indicating that they could also be from humans or canines. Human, canine, and inconclusive coprolites were all analysed for parasites.

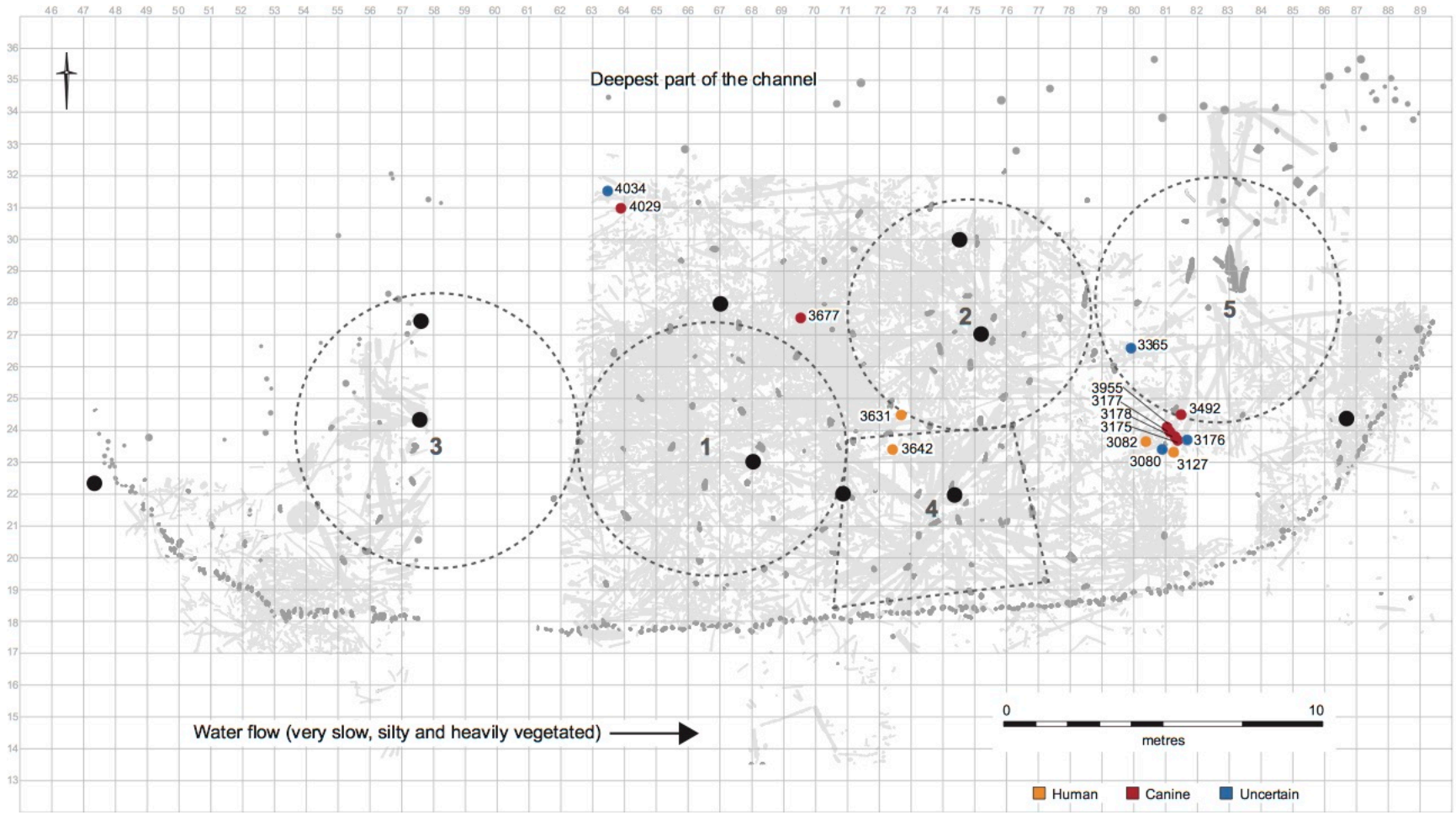

Figure 23: Plan of Must Farm settlement with sediment sampling locations marked by black dots and coprolites marked by coloured circles. From each sediment sampling location around the structures (large dashed circles) samples were collected from three layers (construction/occupation, ash, post-conflagration); for the upstream and downstream locations samples were collected from one layer corresponding to occupation of the site. Human coprolites are marked by yellow circles, canine by red, and inconclusive by blue. Image credit: Vicki Herring, Cambridge Archaeological Unit. (taken from Ledger et al., 2019a)

4.2.1.2 Results

Eggs from five different taxa of helminths were found in the samples studied from the Must Farm settlement. First, in the sediment samples eggs from four taxa were found, namely *Capillaria* sp., *Dioctophyma renale, Diphyllobothrium* sp., and *Trichuris* sp. The distribution and concentrations of these eggs in samples from around the site can be seen in Figure 29.

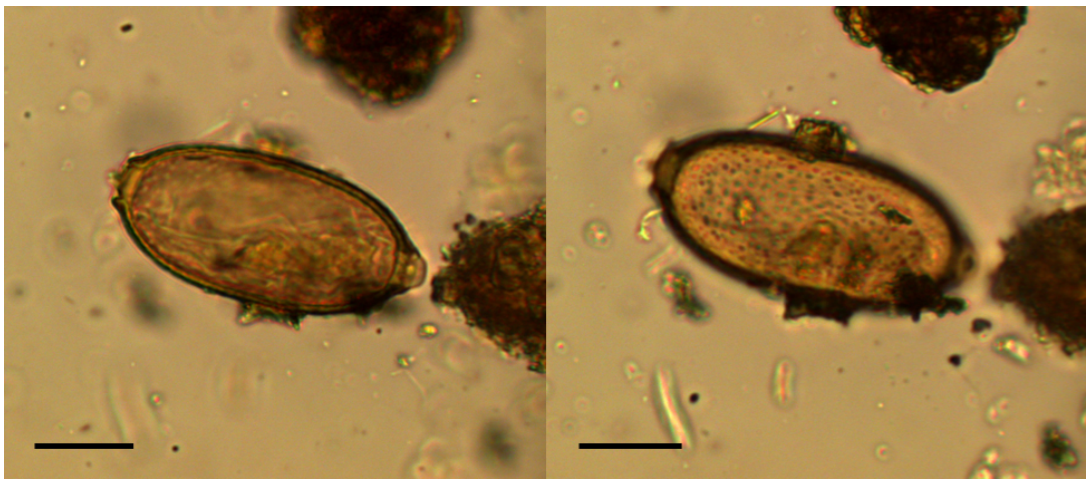

Figure 24: *Capillaria* sp. egg found in a coprolite from the Must Farm settlement. Both images are of the same egg, the image on the right shows the surface structure of the egg. Scale bars are 20 µm.

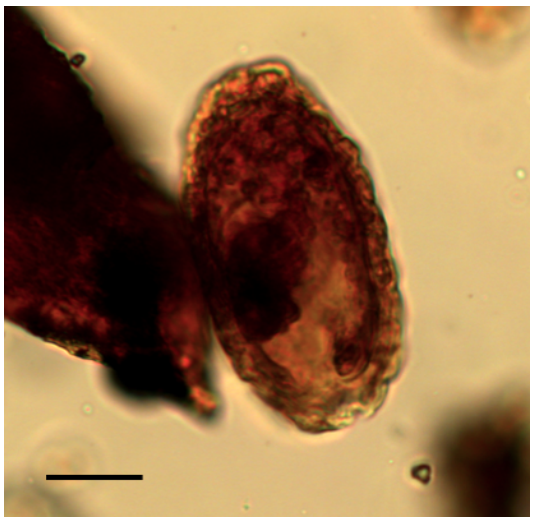

Figure 25: *Dioctophyma renale* egg found in a coprolite from the Must Farm settlement. Scale bar is 20 µm.

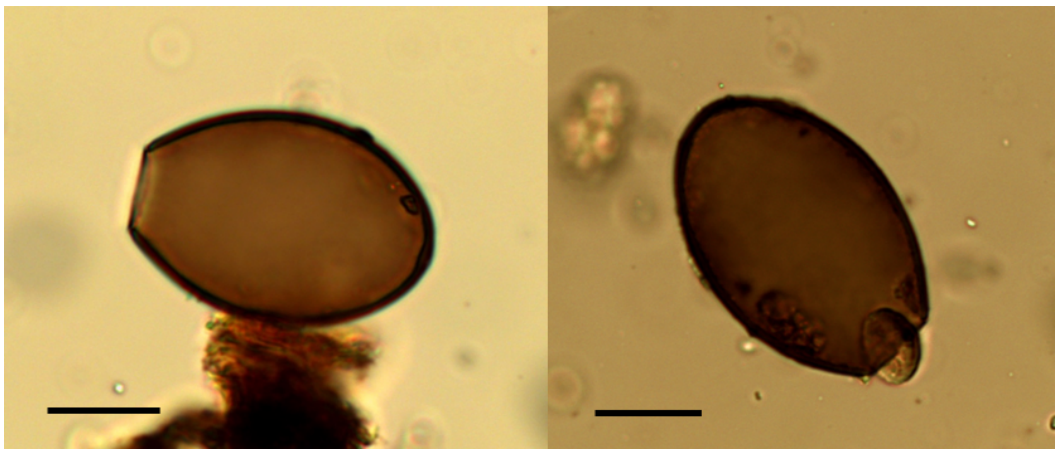

Figure 26: *Diphyllobothrium* sp. eggs found in coprolites from the Must Farm settlement. Scale bars are 20 µm.

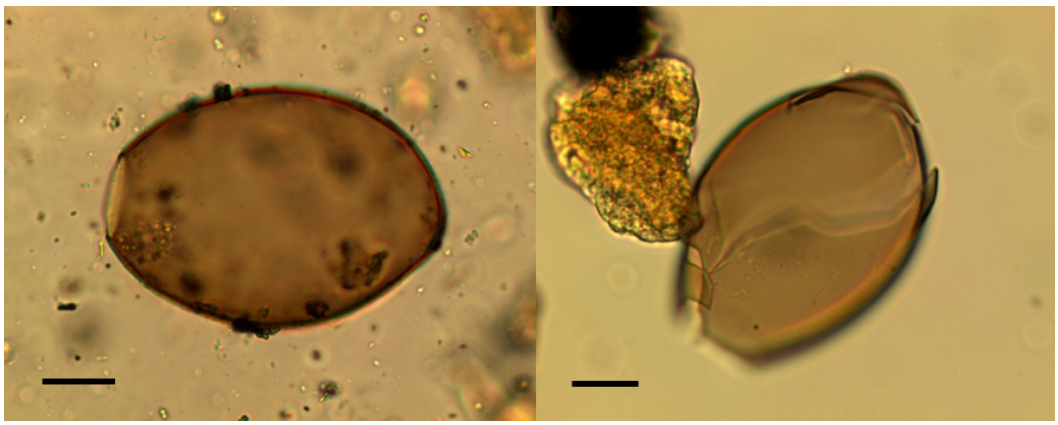

Figure 27: *Echinostoma* sp. eggs found in coprolites from the Must Farm settlement. The egg on the left shows an intact egg with abopercular thickening, many eggs were broken such as the one on the right. Scale bars are 20 µm.

Figure 28: *Trichuris* sp. egg found in a coprolite from the Must Farm settlement. Scale bar is 20 µm.

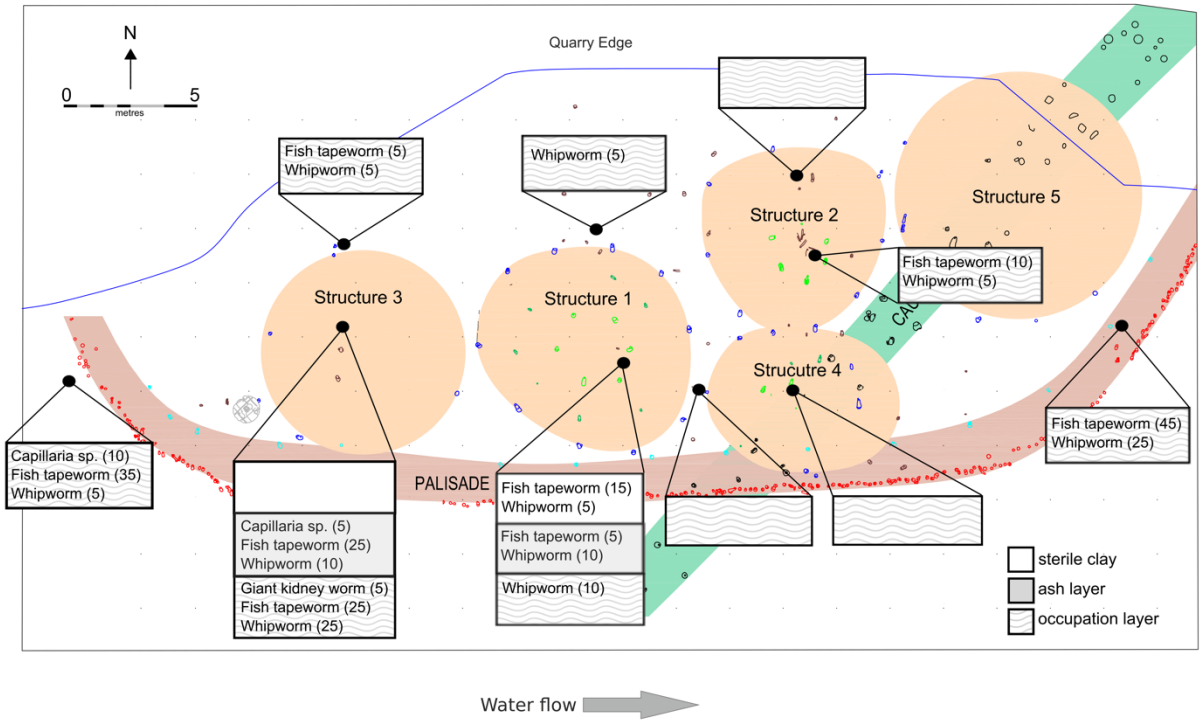

Figure 29: Plan of Must Farm settlement with locations of sediment samples marked by black dots. Boxes indicate taxa found in that layer and concentrations (eggs/gram) of eggs per gram are in brackets; unless no eggs were found then the box is empty.

In the coprolites studied from the Must Farm settlement, five taxa of helminths were found on microscopy. These include *Capillaria* sp. (Figure 24), *Dioctophyma renale* (Figure 25), *Diphyllobothrium* sp. (Figure 26), *Echinostoma* sp. (Figure 27), and *Trichuris* sp. (Figure 28). Fourteen of the fifteen coprolites studied contained helminth eggs, the one coprolite that did not contain any eggs was SF3080. This was one of the coprolites that had an uncertain origin. The same taxa were found in the canine and human coprolites, though a *Dioctophyma renale* egg was found in one of the canine coprolites but no other coprolites.

Capillaria sp. eggs and *Trichuris* sp. eggs have a very similar shape but can be distinguished by the surface structure of the eggs (Moravec, 1980; Bouchet, 1997; Fugassa et al., 2008; Traversa et al., 2011). The *Capillaria* sp. eggs found at Must Farm had a punctuated surface (see Figure 24) consistent with eggs identified in the literature, while *Trichuris* sp. eggs had a smooth surface. Furthermore, the mean length and width of the *Capillaria* sp. eggs was slightly higher than that of *Trichuris* sp. (Table 14), which is consistent with modern size ranges (Mehlhorn, 2016; 381). For further discussion of species identification of eggs from Must Farm see Ledger et al. (2019a).

Table 13: Helminth species and egg concentrations (eggs/g) found in each coprolite studied from the Must Farm settlement.

Coprolite	Host	*Capillaria* sp.	*Dioctophyma renale*	*Diphyllobothrium* sp.	*Echinostoma* sp.	*Trichuris* sp.
SF3080	unknown	-	-	-	-	-
SF3082	human	20	-	-	20	30
SF3127	human	60	-	5	85	195
SF3175	canine	325	-	-	70	5
SF3176	unknown	55	-	-	10	-
SF3177	canine	55	-	-	-	-
SF3178	canine	290	-	-	20	-
SF3365	unknown	20	-	-	10	5
SF3492	canine	615	-	25	15	175
SF3631	human	10	-	-	215	45
SF3642	human	-	-	-	185	125
SF3677	canine	30	-	5	5	20
SF3955	canine	380	-	-	5	-
SF4029	canine	1,750	5	9,415	270	5
SF4034	unknown	1,250	-	11,420	360	10

Table 14: Mean dimensions and standard deviations (SD) of helminth eggs from coprolites and sediment samples from the Must Farm settlement.

Species	Mean Length (µm) ± SD	Mean Width (µm) ± SD
Capillaria sp.	59.9 ± 3.4	31.3 ± 1.5
without polar plugs	59.1 ± 3.1	31.2 ± 1.4
with polar plugs	62.8 ± 2.8	31.4 ± 1.6
Dioctophyma renale	74.9 ± 5.5	40.2 ± 2.1
Diphyllobothrium sp.	58.4 ± 4.7	40.4 ± 2.3
without opercula	57.9 ± 4.6	40.3 ± 2.3
with opercula	61.1 ± 4.5	40.5 ± 2.2
Echinostoma sp.	104.4 ± 8.2	69.9 ± 6.3
without opercula	104.4 ± 8.3	70.0 ± 6.3
with opercula	104.9 ± 7.0	68.1 ± 5.4
Trichuris sp.	54.0	28.3
without polar plugs	53.7	28.2
with polar plugs	58.6	30.0

The ELISA tests run on the sediment samples and coprolites from Must Farm were inconclusive. The first ELISA test was done using sediment samples only. Nine samples were tested. These were the upstream control, the construction/occupation layer from the centre of Structures 1, 2, 3, and 4, the construction layer from outside Structure 3, the post-conflagration layer from inside Structures 1 and 3, and the downstream control. On the first *Cryptosporidium* ELISA test, one positive well out of 8 was found for each of the samples from the construction/occupation layer from the centre of Structure 1, from post-conflagration layer from the centre of Structure 1, and from the downstream control. None of these samples were positive on the re-test. On the first *Entamoeba histolytica* test multiple positive wells were found in samples from the construction/occupation layers from the centre of the four structures, from the upstream control, and from the outer edge of Structure 3. Again none of these positive results were found on the re-test. On the first *Giardia duodenalis* test, positive results were found in multiple wells from all samples except from the post-conflagration sample from Structure 3 and the downstream control, again none of these positive results were found on the second test. For this reason we cannot confidently say that any of these parasites were present in the sediment samples from Must Farm. However, it is possible that the biological repeats were all negative because different subsamples were tested that did not contain the high enough concentrations of the parasite to trigger a positive.

The ELISA tests run on the coprolites were also inconclusive. For the first test, all three ELISA tests were run on SF3127, SF3631, SF3642, SF3082, and SF4029. Due to an inadequate amount of coprolite for a re-test the second ELISA test was run on SF3080, SF3177, SF3642, SF3492, and SF4029. Therefore the only coprolites that were tested twice were SF3642 and SF4029. On the first test nearly all wells were positive for each organism in every coprolite studied. In the second test no coprolites had positive results for *Giardia duodenalis*, each coprolite had positive wells for *Cryptosporidium*, and nearly all wells for each coprolite were positive for *Entamoeba histolytica*. Therefore, the *Giardia duodenalis* results were not confirmed by the second test while the *Cryptosporidium* and *Entamoeba histolytica* results were. However, it was surprising to find positives for every coprolite for all three organisms. The positive and negative control wells produced the proper absorbance values to ensure that the test was performed properly and the ELISA plate and reagents performed as expected. However, so many positive results was very unusual for archaeological samples and combined with the large number of positives on the first run of the sediment samples it is possible that the samples were contaminated with modern waste in the water or that something was cross-reacting with the antibodies in the kits. As such I have recently started a project to look for ancient DNA from these protozoa in the sediment

samples and coprolites to see if we can use a second method to confirm or refute the results and at the same time explore the damage patterns of any DNA recovered to determine if modern contamination may be an issue. All absorbance values from the Must Farm samples can be found in Appendix B.1.

For an in-depth discussion of microscopy results from the Must Farm settlement see Ledger et al., (2019a) (Appendix A.1).

4.2.2 Çatalhöyük

4.2.2.1 Materials

Parasitological analysis of the pelvic soil from Çatalhöyük is published in Antiquity in combination with work done by a previous PhD student, Evilena Anastasiou, who looked for parasite eggs in human coprolites recovered from middens at the site (Ledger et al., 2019b, see Appendix A.2). The samples studied as part of this PhD were the pelvic soil samples from the site.

Çatalhöyük is an early Neolithic site in Turkey, part of which was occupied through to the Chalcolithic. As an early farming community in the Near East this site provides important evidence for parasitic infection with the onset of agriculture. Secondly, the site is one of the best preserved large settlement agglomerations in the Near East. The density of the population at this site was much higher than other Neolithic sites at the time. The settlement structure of the East Mound (7100–6000 cal BCE) consisted of densely packed multi-storey buildings, partially dug into the ground, with little space between them (Bayliss et al., 2015; Hodder, 2014). Archaeological excavations have shown that middens formed directly adjacent to the buildings and these also contained animal and human faeces (Shillito et al., 2011a; Shillito et al., 2011b). Many of the human burials at the site were placed beneath the platforms inside the buildings.

Table 15: Details of burials from Çatalhöyük, from which soil samples were collected from the pelvis of the skeletons (adapted from Ledger et al., 2019b).

Sample Number	Burial ID	Age	Sex	Location	Date	Helminth Eggs
30348.s3	Burial F.7508; Skeleton 30351	20–35 years	Possible female	South Area, Building 43	7100–6700 BC	-
30929.s4	Burial F.3484 Skeleton 30927	9 ± 3 months	Unknown	South Area, Building 89	6700–6500 BC	-
30929.s8	Burial F.3484 Skeleton 30928	35–50 years	Possible female	South Area, Building 89	6700–6500 BC	-
19435.s3	Burial F.3629 Skeleton 8598	20–35 years	Male	North Area, Building 114	6700–6500 BC	-
22027.s5	Burial F.7333 Skeleton 22026	12 ± 2.5 years	Unknown	North Area, Building 77	6700–6500 BC	-
20450.s14	Burial F.3630 Skeleton 19460	11 ± 2.5 years	Unknown	North Area, Building 129	6500–6300 BC	-

The samples studied which were included in this dissertation were pelvic soil samples from six human burials, all of which were found buried inside the houses. The burials date from 7100–6300 BCE. Soil was collected by the excavators, and obtained for analysis in collaboration with Scott D. Haddow and Chirstopher Knüsel. Soil was collected from the pelvis directly anterior to the sacrum and control samples were taken from inside the cranium and around the feet, following standard sampling protocols from our lab (Mitchell, 2017a).

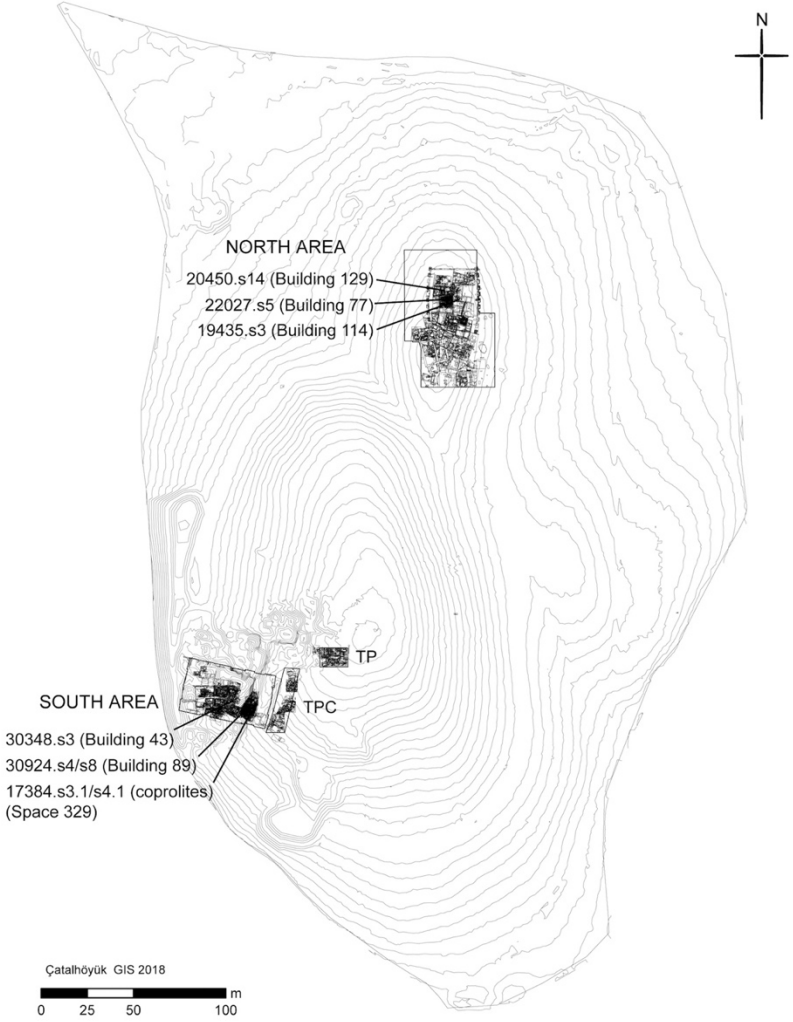

Figure 30: Plan of Çatalhöyük showing location of burials studied and coprolites previously studied by Evilena Anastasiou. Image Credit: Camilla Mazzucato (Ledger et al., 2019b).

4.2.2.2 Results

No helminth eggs were found in the slides studied from the subsamples of pelvic soil from the six individuals studied from Çatalhöyük. As such, and due to the low yields of pelvic soil, these samples were not tested for the presence of protozoan parasites using ELISA. However, previous analysis of human coprolites, done by Evilena Anastasiou, from the site found eggs from *Trichuris trichiura* (Ledger et al., 2019b; Appendix A.2).

4.2.3 Boncuklu

4.2.3.1 Materials

Boncuklu is a neighbouring site to Çatalhöyük in Turkey, located approximately 9.5 km north. Its occupation spans the 9th–8th millennia BCE, slightly earlier than Çatalhöyük (Baird, 2012). Paleoenvironmental reconstructions suggest that the area of the Konya Plain where

Boncuklu is located was a wetland habitat at this time. The site consists of a series of mud-brick structures interspersed by open spaces covering an area of about 1 hectare (Baird et al., 2018). Similar to Çatalhöyük, it represents one of the earliest agricultural societies in this region of the world though inhabitants likely relied on foraging as well. This site has some of the earliest evidence for domestic cereals including emmer wheat. In terms of herding and wild animal consumption, bones of wild boar are the most common animals in the zooarchaeological record, but wild cattle and fish also contributed to the diet of inhabitants (Baird et al., 2018).

Similar to Çatalhöyük, burials were found beneath the floors of the houses. In particular, under floors that have been called 'clean' areas of the houses as opposed to 'dirty' areas that contained hearths and served as a kitchen areas. Soil from the pelvis of skeletons excavated during the 2014 and 2015 field seasons was analysed. These soils were obtained in collaboration with the director of the site, Doug Baird. Samples were collected by the excavators from directly on top of the sacrum and control samples were also collected from inside the cranium and around the feet where possible. Samples were collected from 9 individuals (Table 16).

Table 16: Details of burials from Boncuklu, from which soil samples were collected from the pelvis of skeletons.

Context Number	**Sample ID**	**Samples Collected**	**Helminth Eggs in Pelvic Soil**
ZHCB	MS476 Grave 40	Pelvis and control from cranium	-
ZHCB	MS 478 Grave 40	Pelvis and control from cranium	-
ZPV	MS 448	Pelvis and control from cranium and hand area	-
ZPS	MS 444 Grave 38	Pelvis and control from feet	-
MMVW	MS 479 Grave 39	Pelvis and control	-
MMVO	MS 539	Pelvis	-
MMXC	MS588 Grave 46	Pelvis and control from cranium	-
ZJB	MS552	Pelvis and control from cranium	-
ZJB	MS608 Grave 48	Pelvis and control from cranium	-

4.2.3.2 Results

No helminth eggs were found in the slides studied from the subsamples of pelvic soil from the nine individuals sampled from Boncuklu. As such, and due to the low yields of pelvic soil these samples were not tested for the presence of protozoan parasites using ELISA.

4.2.4 Griffin Warrior, Palace of Nestor, Pylos

4.2.4.1 Materials

The Griffin Warrior was a Mycenaean individual from Greece. This was the burial of a male, 30–35 years old at the time of death, who was buried in a shaft grave near the Palace of Nestor, Pylos (Davis and Stocker, 2016). The grave good assemblage exemplified the wealth and status of this individual and included numerous weapons, jewellery, and metal vessels which led to the name and identification of this male as a warrior with high status in the community. Ceramic fragments in the fill of the tomb and grave goods were used to date the burial to the Late Helladic IIA period (1450–1400 BCE). The tomb itself consisted of a shaft cut into the bedrock that was lined with stones. The body of the Griffin Warrior was placed in a wooden coffin inside this grave. The tomb of the Griffin Warrior was located near Tholos Tomb IV, which was likely in use during the Middle Bronze Age, slightly earlier than the burial of the Griffin Warrior (Davis and Stocker, 2016).

The nearby Palace of Nestor is located in the western Peloponnese in Pylos, and is one of the best preserved palaces of its time. It is thought to have been at its height during the Early Mycenaean period and would have been inhabited by local dynasty, the Neleid family, the most famous of whom was Nestor. The palace was destroyed sometime near the end of the 13th c. BCE when it was abandoned (Blegen and Rawson, 1967).

Soil taken from the pelvic area of the individual was studied for this work, this was collected by the excavators of the grave under the direction of Sharon Stocker, Jack Davis, and Lynne Schepartz. Control samples from the cranium and above the burial were also collected.

4.2.4.2 Results

No helminth eggs were found in the slides studied from the subsamples of the Griffin Warrior pelvic soil. The sample was not tested for protozoan parasites using ELISA due to the low yield of pelvic soil and the high cost of the tests.

4.3 Roman Sites

4.3.1 West Smithfield, London

4.3.1.1 Materials

The cemetery to which the burials from West Smithfield belong is referred to as the Roman Western Cemetery by the Museum of London, UK. The cemetery is located to the west of the Roman city of Londinium (modern-day London). The cemetery is thought to have been located along a road connecting London to Silchester. The excavations of West Smithfield (1–4 Giltspur Street) are one of many excavations that are all part of the same cemetery (Redfern and Mikulski, 2009). The earliest burials from the cemetery date to the 1st c. CE and it was used into the early 5th c. CE. Demographic analysis of skeletal remains from the site suggest that the cemetery was used for inhabitants of Roman period London. Individuals from all age groups, except those <1 year were observed and both males and females are represented (Redfern and Mikulski, 2009).

From the West Smithfield excavations 29 individuals were sampled during excavations for parasitological analysis. These samples were obtained from collections at the Museum of London in collaboration with Rebecca Redfern. As with other sites, soil was collected from the pelvis directly on top of the sacrum and control samples came from above or below the skeleton in most cases (Table 17). In addition, one Medieval period coprolite was also collected and analysed (Context 311).

Table 17: Details of burials from West Smithfield Cemetery, London from which soil samples were collected from the pelvis of skeletons.

Context Number	**Age**	**Sex**	**Samples Collected**	**Helminth Eggs**
228	12–17 years	Undetermined	Pelvis and control from above skeleton	-
311	N/A	N/A	Medieval coprolite	*Ascaris* sp.
467	36–45 years	Male	Pelvis and control from above skeleton	-
485	Adult	Male	Pelvis and control from above skeleton	-
493	Adult	Undetermined	Pelvis and control from above skeleton	-
504	26–35 years	Female	Pelvis and control from above skeleton	-
512	36–45 years	Male	Pelvis	-
527	6–11 years	Undetermined	Pelvis and control from above skeleton	-
558	Adult	Undetermined	Pelvis	-

570	Adult	Undetermined	Pelvis and control from above skeleton	-
579	Adult	Undetermined	Pelvis (sample 36) and control from above skeleton	-
579	Adult	Undetermined	Pelvis (sample 43) and control from above skeleton	-
599	36–45 years	Female	Pelvis and control from above skeleton	-
611	Adult	Intermediate	Pelvis	-
656	Adult	Female	Pelvis	-
660	12–17 years	Undetermined	Pelvis and control from above skeleton	-
686	36–45 years	Male	Pelvis and control from above skeleton	-
691	18–25 years	Female	Pelvis and control from below skeleton	-
761	Adult	Male	Pelvis and control from above skeleton	*Ascaris* sp.
781	6–11 years	Undetermined	Pelvis	-
785	Subadult	Undetermined	Pelvis and control from below skeleton	-
837	Adult	Male	Pelvis and controls from head and above skeleton	-
841	Adult	Intermediate	Pelvis and control from above skeleton	*Ascaris* sp.
848	12–17 years	Undetermined	Pelvis and control from above skeleton	-
870	Adult	Female	Pelvis and control from above skeleton	-
877	Adult	Intermediate	Pelvis and control from above skeleton	-
885	Adult	Male	Pelvis and control from above skeleton	-
915	18–25 years	Intermediate	Pelvis and control from below skeleton	-
935	Adult	Female	Pelvis and control from above skeleton	*Ascaris* sp.
1004	6–11 years	Undetermined	Pelvis	-
1052	Adult	Female	Pelvis and control from above skeleton	-

Note: Age and sex data provided by Rebecca Redfern, Museum of London. All burials are Roman except Context 311 which is a Medieval coprolite.

4.3.1.2 Results

Helminth eggs were found in the pelvic soil of three of the 29 individuals studied from the cemetery of West Smithfield in London. Only *Ascaris* sp. eggs were found in all three individuals (Figure 31). The positive individuals were 761 (Adult Male), 841 (Adult, Undetermined Sex), and 935 (Adult Female). The egg concentrations were low in the pelvic soil samples. Five eggs were found in the 0.2g subsample from individual 761, three in individual 841, and two in individual 935. The egg concentrations and mean dimensions of

these eggs are listed in Table 18. No helminth eggs were found in the control samples from the three individuals, the entire 0.2g subsample was analysed for the control samples.

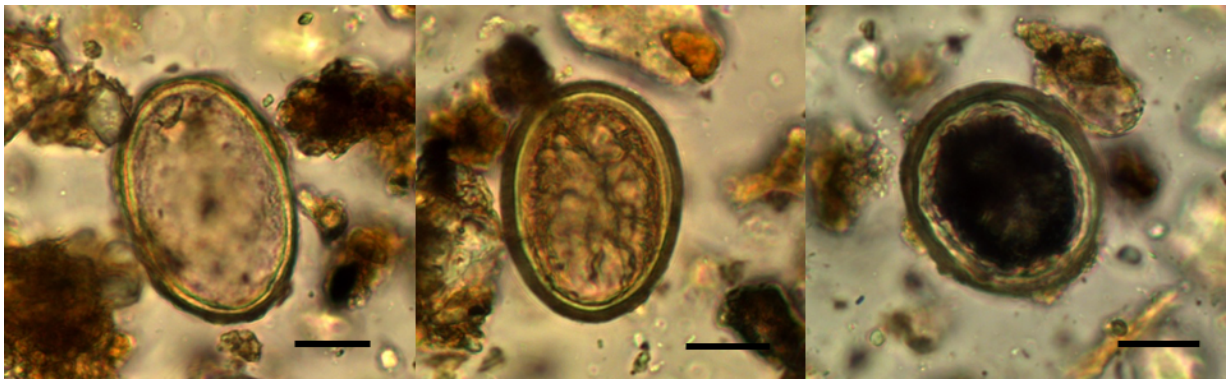

Figure 31: *Ascaris* sp. eggs found in pelvic soil from burials at West Smithfield cemetery, London. Scale bars are 20µm.

Table 18: Mean length, width, standard deviations (SD), and egg concentrations (eggs/g) of *Ascaris* sp. found in pelvic soil from West Smithfield.

Context Number	Species	Egg Concentration	Mean Length (μm) ± SD	Mean Width (μm) ± SD
761	*Ascaris* sp.	25	58.0 ± 3.2	46.1 ± 2.6
841	*Ascaris* sp.	15	52.3 ± 8.6	44.6 ± 6.3
935	*Ascaris* sp.	10	59.5 and 68.8	45.2 and 45.7
Medieval Coprolite	*Ascaris* sp.	55	58.7 ± 4.1	45.3 ± 2.4

Note: For Context 935 only two eggs were found so their dimensions are listed without a calculated mean.

In addition, *Ascaris* sp. eggs were also found in the medieval coprolite recovered in the cemetery excavations. A higher concentration of eggs was found in the coprolite compared to the pelvic soil from Roman individuals (see Table 18).

ELISA analysis was not used to test for protozoan parasites in these samples due to the low yield of pelvic soil and high cost of the tests.

4.3.2 Winterborne, Dorset

4.3.2.1 Materials

Additional pelvic soil samples from Roman Britain were studied from the site of Winterborne, Dorset in southwest Britain. This area was occupied by a tribe known as the Durotriges in the

Iron Age until Britain came under control of the Roman Empire (Russell et al., 2014). Previous excavations on the site focused on an Iron Age banjo enclosure that was used between the 2nd c. BCE and 3rd c. CE., though its use declined in the 1st c. CE. Banjo enclosures are a feature found on multiple Iron Age sites in Britain. They typically consist of a sub-circular enclosure with an elongated passageway thought to serve as an entrance, they are thought to be occupation areas (McOmish, 2018). Artefacts found at the banjo enclosure and associated settlement included both Durotrigian and Roman items indicating the continual use of the site through this transitional period. This makes it an interesting site to consider the influence of pre-Roman populations on Roman settlements.

Southwest of the banjo there was a small Roman villa with other features around it that may have been houses (Russell et al., 2015). The villa was built around 320 CE and the area occupied up to the 5th c. CE. A number of ditches were excavated from near the villa one of which contained the burials of five Roman individuals; two adult males and three adult females. Pelvic soil from these five individuals was collected by the excavators, under the direction of Martin Smith, for analysis (Table 19). The burials are all Roman, dated to the 4th c. CE.

Table 19: Details of burials from Winterborne, Dorset from which soil samples were collected from the pelvis of skeletons.

Burial Number	Skeleton Number	Samples Collected	Helminth Eggs in Pelvic Soil
1073	1167	Pelvis and controls from cranium and feet	-
1075	1164	Pelvis and controls from cranium and feet	-
1077	1165	Pelvis and controls from cranium and feet	-
1079	1166	Pelvis and controls from cranium and feet	-
1107	1168	Pelvis	-

4.3.2.2 Results

No helminth eggs were found in the subsamples of pelvic soil from the five burials at Winterborne, Dorset. ELISA analysis was not used to test for protozoan parasites in these samples due to the low yield of pelvic soil and high cost of the tests.

4.3.3 Lauriacum

4.3.3.1 Materials

Lauriacum is a Roman site in Austria located on the Enns river. Lauriacum was located on the Danube limes, in the province of *Noricum*, and was an important legionary fortress. The Roman *limes* in the province of Noricum were built along the Danube river starting in the 1st c. CE with major legionary bases between those at Vienna and Carnuntum (Jilek et al., 2011). After the Marcomannic wars (166–180 CE) a new legion was placed at Lauriacum. It was located along roads into Germany and from Italy and Asia Minor (Ubl, 2002). The city was built up around its legionary fortress located between the river Ens and Danube, which was established in the 2nd c. CE. There is some evidence for a small *vicus* (village) around the 1st c. CE but the larger civilian settlements were built in the 2nd c. CE (Ubl, 2002). The settlement at Lauriacum was typical of other civilian towns along the *limes*. It consisted of residential areas (*insulae*) some of which had painted walls and mosaic floors, there are also workshops, small gardens and cemeteries. The population was diverse likely consisting of recruited soldiers from local areas and farther regions of the empire, their families, and merchants. The city of Lauriacum was destroyed during invasions of Germanic tribes in the early 3rd c. CE, after which it was rebuilt. The city appears to have been abandoned in the 5th c. CE after invasion by the Huns.

The burials studied here come from one of the cemeteries found at the site, the cemetery of Steinpass. The burials mostly date between the 3rd–4th c. CE. Pelvic soil was collected from thirteen individuals housed in the Oberösterreichisches Landesmuseum collections. Soil was collected from the skeletal collection by Maria Marschler. Soil present in the sacral foramina was collected for analysis. Soil present in the cranium of these individuals was collected as a control.

Table 20: Details of burials from Lauriacum, Austria from which soil samples were collected from the pelvis of skeletons.

Grave Number	Individual	Age	Sex	Samples Collected	Helminth Eggs in Pelvic Soil
147	1	21–25 years	Female	Pelvis and control from cranium	-
230	N/A	30–40 years	Male	Pelvis and control from cranium	-
273	1	18–22 years	Male	Pelvis and control from cranium	-
282	N/A	20–30 years	Male	Pelvis and control from cranium	-
284	N/A	40–60 years	Male	Pelvis and control from cranium	-
290	N/A	20–25 years	Indeterminate	Pelvis and control from cranium	-
291	N/A	20–25 years	Female	Pelvis and control from cranium	-
293	N/A	6–9 years	Undetermined	Sacrum and control from cervical vertebrae	-
301	N/A	18–22 years	Female	Pelvis and control from cranium	-
308	1	35–50 years	Male	Pelvis and control from cranium	-
343	N/A	25–40 years	Female	Pelvis and control from cranium	-
363	N/A	20–25 years	Male	Pelvis and control from cranium	-
368	1	25–40 years	Female	Pelvis and control from cranium	-

Note: Age and sex data was collected by Maria Marschler.

4.3.3.2 Results

No helminth eggs were found in the subsamples of pelvic soil from the 13 burials sampled from Lauriacum. ELISA analysis was not used to test for protozoan parasites in these samples due to the low yield of pelvic soil and high cost of the tests.

4.3.4 Arlon

4.3.4.1 Materials

Arlon, or *Orolauno*, was a Roman *vicus* in modern-day Belgium, the Roman province of *Belgica*. It flourished between the 1st–3rd c. CE, and is thought to have had an economy reliant on various crafts as established by the many workshops that have been excavated from the site. These workshops include those for iron metallurgy, wood turning, leather processing, and glass making (Defgnée et al., 2008). Around the 4th c. CE a military fortress was built on the *vicus* covering an area of about 5 hectares (Pigière and Henrotay, 2012). It was located along two Roman roads, one from Reims to Trier and one from Metz to Tongeren (Pigière and Henrotay, 2012).

Numerous buildings have been identified that are thought to be workshops for different types of craft. One of these has been analysed fairly extensively, it is a large building with good water supply, and numerous vats and pits. Sediment from the pipes and vats underwent archaeobotanical analysis and at the time helminth eggs were also identified. These eggs were identified as roundworm (*Ascaris* sp.) and whipworm (*Trichuris* sp.) (Defgnée et al., 2008). The organization of the building and associated analysis led to identification of the building as a fuller's workshop. As urine from animals and in some cases humans is often used in the fulling process, it is unclear if these eggs were from humans or animals. It is possible that the reused urine was contaminated with faecal material and could easily contain some helminth eggs.

The samples studied as part of this research came from the fill of a Roman period latrine (F05) at the site that dates between 250–280 CE. The area of the site where the latrine was found was excavated in 2003–2006 by the archaeological heritage agency of the Walloon region and samples were obtained from a collaboration with Koen Deforce. The latrine was found while excavating an artisanal and residential quarter of the *vicus*. As this latrine was primarily for human use, this context should help us clarify if roundworm and whipworm were infecting humans living at the Arlon *vicus*.

4.3.4.2 Results

Eggs from *Ascaris* sp. (roundworm) and *Trichuris* sp. (whipworm) were found in the subsample studied from the Roman latrine at Arlon, Belgium (Figure 32 and Figure 33), as well as two eggs each of *Capillaria* sp. and *Dicrocoelium dendriticum.* These eggs were well preserved, as indicated by the presence of a mamillated coat on most of the roundworm eggs

which is often lost in archaeological samples. The concentration of roundworm eggs in the sample was 990 eggs/gram and the concentration of whipworm was 1,795 eggs/g. The majority of roundworm eggs found were fertile eggs though far fewer infertile eggs were present as well. The mean length of fertile roundworm eggs was 66.7 µm, and the mean width was 51.9 µm. For infertile eggs, the mean length was 84.0 µm and the mean width was 47.6 µm. The dimensions of whipworm eggs are presented in Table 21 for eggs with and without polar plugs. The mean length of whipworm eggs with both polar plugs was 52.9 µm, with one polar plug 51.9 µm, and with no polar plugs 50.3 µm. The mean width of all whipworm eggs was 26.7 µm. There is a large overlap in the size ranges of *T. trichiura* and *T. suis*, however, as can be seen in Figure 34 most of the eggs found in the Arlon latrine are clustering in the size range of human whipworm (*T. trichiura*). In conjunction with this, these eggs were found in a latrine used by humans so they are most likely from the human-infecting species *T. trichiura*.

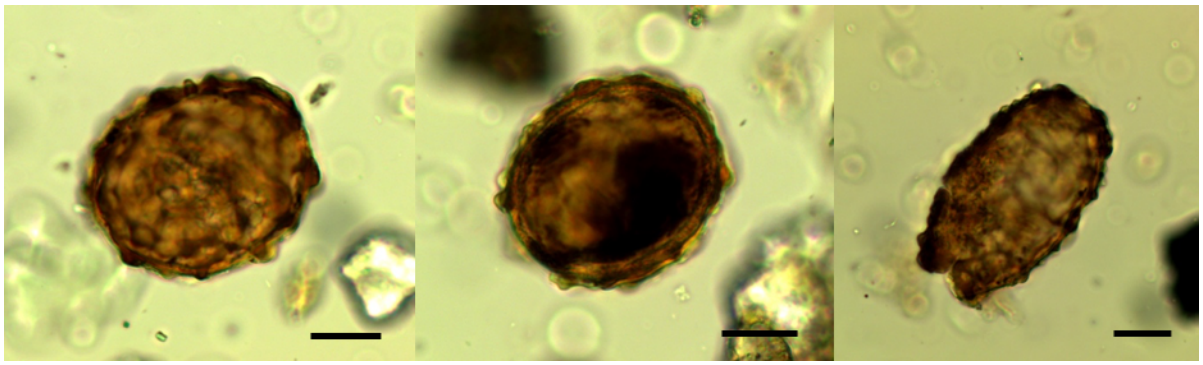

Figure 32: *Ascaris* sp. eggs from Arlon latrine. Fertile eggs with mamillated coats (left and centre), infertile egg (right). Scale bars are 20µm.

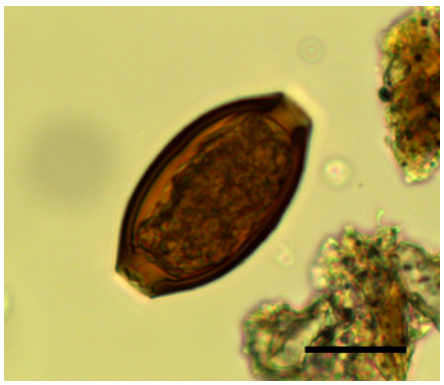

Figure 33: *Trichuris* sp. egg from Arlon latrine. Scale bar is 20µm.

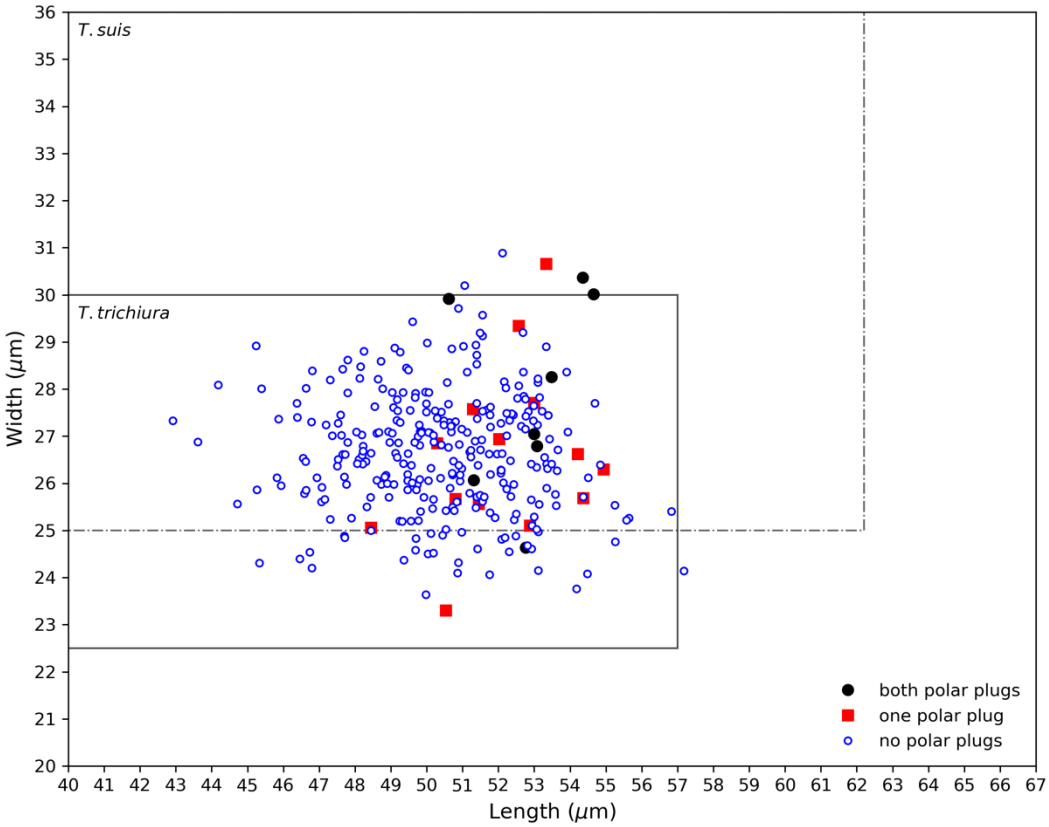

Figure 34: Dimensions of *Trichuris* sp. (whipworm) eggs from the Arlon latrine plotted against reported dimensions of *T. trichiura* eggs (solid box) and *T. suis* eggs (dashed box) without polar plugs (Beer, 1976). Eggs without polar plugs are marked by open blue circles, eggs with one polar plug are marked by red squares, and eggs with both polar plugs are marked by black circles.

In addition to roundworm and whipworm, two eggs of *Capillaria* sp. were found and two eggs of *Dicrocoelium dendriticum* were found (Figure 35 and Figure 36). Therefore, the concentration of both *Capillaria* sp. and *Dicrocoelium dendriticum* eggs was 10 eggs/g. *Capillaria* sp. eggs were differentiated from *Trichuris* sp. based on the characteristic pitted surface of *Capillaria* sp., while *Trichuris* sp. eggs have smooth egg shells. Both *Capillaria* sp. eggs were missing their polar plugs. The length of the eggs was 49.3 µm and 58.4 µm, and the corresponding widths were 30.3 µm and 24.4 µm. Both *Dicrocoelium* eggs found had their opercula still attached. The length of the eggs was 42.3 µm and 40.1 µm, and the corresponding widths were 24.9 µm and 22.5 µm. These eggs are most likely from *D. dendriticum* as this is the species that is the most common in Europe, while *D. hospes* is found mainly in Africa and *D. chinensis* in East Asia (Otranto and Traversa, 2002; Mehlhorn, 2016).

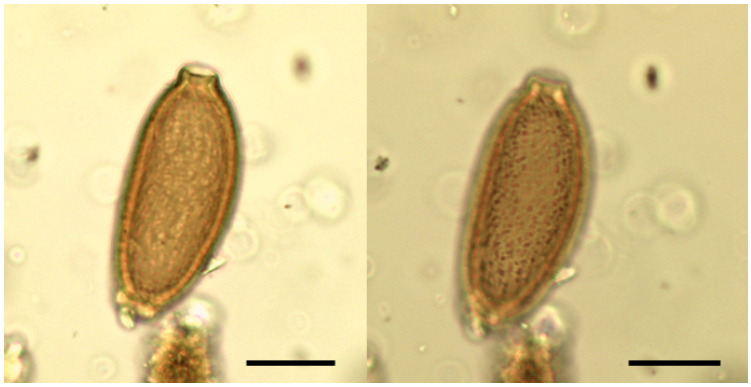

Figure 35: *Capillaria* sp. egg from the Arlon latrine. The images are of the same egg, the surface structure of the egg is visible in the image on the right. Scale bars are 20µm.

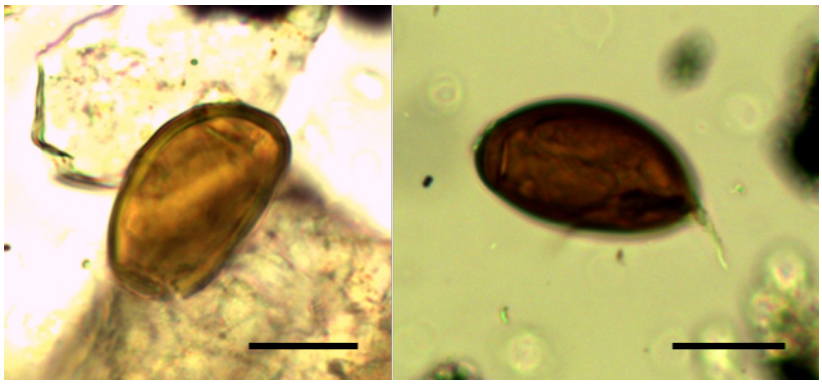

Figure 36: *Dicrocoelium dendriticum* eggs from the Arlon latrine, both with attached opercula. Scale bars are 20µm.

Table 21: Details of eggs found in the latrine sample from Arlon. Egg concentrations are in eggs/g and mean dimensions are presented for each species for eggs that could be measured.

Species	Number of eggs	Eggs/g	Mean Length ± SD (µm)	Mean Width ± SD (µm)
Ascaris sp.	198	990		
fertile	155	775	66.7 ± 4.9	51.9 ± 4.2
infertile	11	55	84.0 ± 6.2	47.6 ± 4.4
Capillaria sp. (no polar plugs)	2	10	53.8 ± 6.4	27.3 ±4.2
Dicrocoelium dendriticum (with opercula)	2	10	41.2 ± 1.5	23.7 ± 1.7
Trichuris sp.	359	1,795	50.5 ± 2.4	26.7 ± 1.3
both polar plugs	10	50	52.9 ± 1.2	27.9 ± 2.1
one polar plug	16	80	51.9 ± 2.1	26.6 ± 1.9
no polar plugs	299	1,495	50.3 ± 2.3	26.6 ± 1.3

Notes: Dimensions in shaded rows are for all eggs that could be measured. To calculate concentrations all eggs were counted even if they could not be measured.

The first ELISA test produced one positive well out of eight for *Giardia duodenalis*. However, on the repeat test with a different subsample there were no wells with positive results for *Giardia duodenalis*, thus we cannot confidently say that *Giardia* is present in the sample. However, it is possible that the parasite was present in very low concentrations and thus was only detected in one of 8 technical repeats on the first test and not detected in the second subsample. All tests were negative for *E. histolytica* and *Cryptosporidium*. The absorbance values for the ELISA tests can be found in Appendix B.2.

4.3.5 Paphos

4.3.5.1 Materials

Paphos is an ancient city in Cyprus, occupied from the Neolithic period onwards. All samples studied here are from the Hellenistic/Roman period. Pelvic soil from individuals buried in three Hellenistic-Roman tombs excavated from the centre of the modern city was collected for analysis. These were part of rescue excavations by the Department of Antiquities of Cyprus. A total of six tombs were excavated though samples could only be collected from three. These tombs are thought to be part of a larger cemetery located 3.5 km north of the ancient city of Nea Paphos, which was the capital of Cyprus during the Hellenistic and Roman periods. They were rock-cut tombs, typical of this period in Cyprus, with a dromos leading into a central chamber that had a series of niches in the walls. Most tombs had multiple individuals, buried in the niches or in the centre of the central chamber. Some individuals were placed in sarcophagi.

Soil from the pelvis of six skeletons was collected by the excavators for analysis, these samples were obtained from a collaboration with Kirsi Lorentz and Grigoria Ioannou. These six individuals were buried in tombs MP3937, MP3938, and MP3939. Four individuals were sampled from tomb MP3937, all were buried in the central chamber. They were all supine in the extended position. The individual sampled from tomb MP3938 was buried in one of the niches, supine in the flexed position. Finally the individual from tomb MP3939 was buried in a sarcophagus in the central chamber. Samples were collected from the pelvis directly anterior to the sacrum and, where possible, controls were taken from inside the cranium and around the feet.

Table 22: Details of burials from Paphos, Cyprus from which soil samples were collected from the pelvis of skeletons.

Tomb Number	Skeleton Number	Age	Sex	Samples Collected	Helminth Eggs in Pelvic Soil
MP3937	SK1	44–51 years	Male	Pelvis and controls from cranium and feet	-
MP3937	SK2	30–34 years	Male	Pelvis	-
MP3937	SK3	30–39 years	Male	Pelvis	-
MP3937	SK4	40–44 years	Female	Pelvis and control from feet	-
MP3938	SK1	Adult	Undetermined	Pelvis and control from feet	-
MP3939	SK1	40–44 years	Male	Pelvis and controls from cranium and feet	*Ascaris* sp.

Note: Age and sex data for each individual was provided by Grigoria Ioannou.

4.3.5.2 Results

Parasite eggs were recovered from the pelvic soil of one individual, skeleton 1 in tomb MP3939, out of the six individuals studied from Paphos. This was the individual buried in the sarcophagus. No parasite eggs were found in the 0.2g control subsample from the cranium or feet of this individual. No parasite eggs were found in the pelvic soil samples from any other individuals.

Two roundworm (*Ascaris* sp.) eggs were found in the subsample of pelvic soil from the individual buried in the sarcophagus (Figure 37). This gives an egg concentration of 10 eggs/g. The dimensions of the two eggs was 57.7 µm long and 46.3 µm wide for the first, and 59.6 µm long and 45.3 µm wide for the second. These dimensions fit into the typical size range of fertile roundworm eggs which is 45–75 µm long and 35–50 µm wide (Garcia, 2016). Both eggs were decorticated, meaning they had lost their outermost mamillated coat. As a result, these eggs would be expected to fall on the lower end of the typical size ranges.

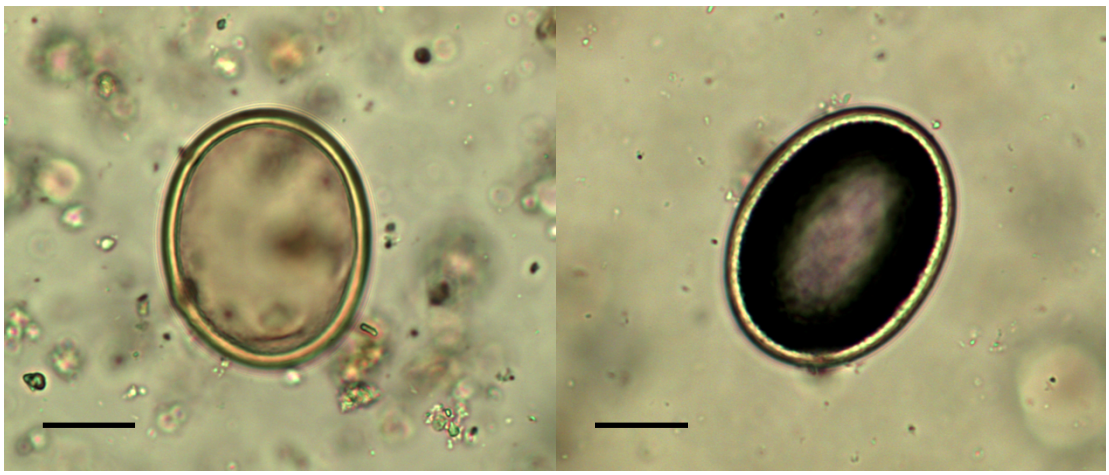

Figure 37: *Ascaris* sp. eggs from the pelvic soil of skeleton 1, in the sarcophagus of MP3939 at Paphos. Scale bars are 20µm.

Recovery of roundworm eggs in the pelvic soil from the individual buried in the sarcophagus, in the absence of any eggs in control samples collected from the cranium and the feet, suggests that this individual did have a true infection. In the case of the individual buried in the sarcophagus it is likely that this represents a true infection as the individual was buried in a closed environment, the sarcophagus. This makes it less likely for eggs from contaminated soil to be introduced, for example as might happen when a body buried in the ground has soil from the surface contaminated with parasite eggs thrown over the body when filling in the grave. When studying samples collected from latrines, egg concentrations are often much higher as we have the chance to find helminth eggs from many people who used the latrine over an extended period of time, making this a location where helminth eggs can accumulate. On the other hand in the case of pelvic soil we will only detect helminth eggs that were present in the individual's intestines at the time of death. Therefore, the low concentration of 10 eggs/g could still represent an infection in this individual.

ELISA analysis was not used to test for protozoan parasites in these samples due to the low yield of pelvic soil and high cost of the tests.

4.3.6 Oplontis

4.3.6.1 Materials

Oplontis, located in the modern city of Torre Annunziata, is a Roman site in the Bay of Naples, Italy. The site was destroyed by the eruption of Mount Vesuvius in 79 CE, similar to Pompeii. At the time of occupation, the villa would have been located on the coast of the Bay of Naples and shares similarities with other villas found in the Italian countryside of

Campania. There are two main building areas at the site, referred to as Villa A and Villa B. Villa A is an elite villa that would have been built by and visited by wealthy Romans. Villas such as these in the Italian countryside served as vacation homes as well as a way to display ones social status and maintain social and political obligations (Gazda, 2014). Villa A at Oplontis consists of opulently decorated rooms and gardens for hosting guests, dining, leisure, as well as service areas for slaves who would have worked at the villa. Villa B on the other hand, is not in true sense a villa as has been made clear by further excavation, it is actually thought to be a commercial area for shops and trade or distribution of goods, with some living areas (Gazda and Clarke, 2016). Villa B consists of a central courtyard surrounded by two-storey buildings with more than 70 rooms (Thomas, 2016; Van der Graaff et al., 2016). Some of these rooms contained large numbers of shipping jars (amphorae) which were used to transport wine and fish sauce attesting to the trade connections of the villa (Muslin, 2016). Furthermore, the opulent materials used to decorate some of the upper floor rooms can be traced to various locations. For example, marble quarried from Greece, Tunisia, and Egypt was found in the residential areas (Muslin, 2016).

In one of these rooms, room 10, the skeletal remains of 54 individuals were discovered (Ward, 2016) and these were recently excavated in 2017 by Kristina Killgrove and Nicola Terrenato (**Error! Reference source not found.**). It seems likely that the individuals found in this room were residents of Oplontis and possibly refugees from the surrounding area hiding out during the eruption of Vesuvius. Some scholars have suggested that a subset of the individuals who were recovered with jewellery and other precious finds were wealthy individuals and even potentially the owner of the villa and his family, while the others may have been servants or slaves (Ward, 2016). As some individuals had been exposed since their first discovery it was not possible to collect soil samples from the pelvis of all skeletons but samples were collected by the excavators, under the direction of Kristina Killgrove, from eight undisturbed individuals (Table 23). These individuals died during the eruption of Mount Vesuvius.

Table 23: Details of burials from Oplontis, Italy from which soil samples were collected from the pelvis of skeletons.

Skeleton ID	Age	Sex	Samples Collected	Helminth Eggs in Pelvic Soil
C	Middle Adult	Female	Pelvis and control from cranium	-
D	Young Adult	Female	Pelvis and control from cranium	*Ascaris* sp., *Trichuris trichiura*
E	Middle Adult	Female	Pelvis and control from cranium	-
I	Adolescent	Indeterminate	Pelvis and control from cranium	*Ascaris* sp.
J	Middle Adult	Female	Pelvis and control from cranium	-
K	Young Adult	Female	Pelvis and control from cranium	-
M	Young Adult	Female	Pelvis and control from cranium	-
O	Older Child	Indeterminate	Pelvis and control from cranium	-

Note: Age and sex data provided by Kristina Killgrove.

4.3.6.2 Results

Helminth eggs were found in the pelvic soil from two of the eight individuals sampled from Oplontis. In Individual D eggs from *Ascaris* sp. and *Trichuris trichiura* were found (Figure 38 and Figure 40). In Individual I only eggs from *Ascaris* sp. were found. The control samples from the cranium of these two individuals did not contain any parasite eggs. No other individuals had helminth eggs in the subsample of soil studied.

In Individual D a total of 197 *Ascaris* sp. eggs were found in the 0.2 g subsample studied, giving a concentration of 985 eggs/g. All of the *Ascaris* sp. eggs were fertile and decorticated – missing their mamillated coats. The mean length of *Ascaris* sp. eggs was 55.8 µm and the width was 44.4 µm. Six *Trichuris trichiura* eggs were found giving a concentration of 30 eggs/g. None of the eggs had either polar plug present. The mean length of *T. trichiura* eggs was 44.9 µm and width was 24.3 µm. This fits within the standard size range for *T. trichiura* eggs missing their polar plugs and is not in the overlapping range with *T. suis* (Beer, 1976).

In Individual I only *Ascaris* sp. eggs were found. All were fertile and decorticated similar to Individual D. The concentration of eggs found was 45 eggs/g with the mean length 56.3 µm and width 43.6 µm.

Table 24: Dimensions and egg concentrations of helminths found in pelvic soil from Oplontis.

Individual	Species	Number of eggs	Eggs/g	Mean Length ± SD (*µ*m)	Mean Width ± SD (*µ*m)
D	*Ascaris* sp.	197	985	55.8 ± 3.4	44.4 ± 2.0
	Trichuris trichiura	6	30	44.9 ± 2.1	24.3 ± 1.2
I	*Ascaris* sp.	9	45	56.3 ± 3.4	43.6 ± 2.2

The eggs identified as *Ascaris* sp. eggs in the pelvic soil from Oplontis are the proper shape and size however, their initial identification was uncertain do the irregularity of the surface of the egg shell. The surface of the shell appears to be slightly porous or spiculated (Figure 39) while typically decorticated roundworm eggs are smooth. For this reason images of the eggs were sent to a number of other specialists to ensure that they were not misidentified. Collaborators from Oplontis and Italy more widely have confirmed that they are not pollen, fungal spore, or other non-pollen palynomorphs thus the most likely explanation still seems to be that they are *Ascaris* sp. eggs. It is possible that the high heat and ash environment that they were exposed to during the eruption of Mount Vesuvius resulted in the irregular appearance of the eggs.

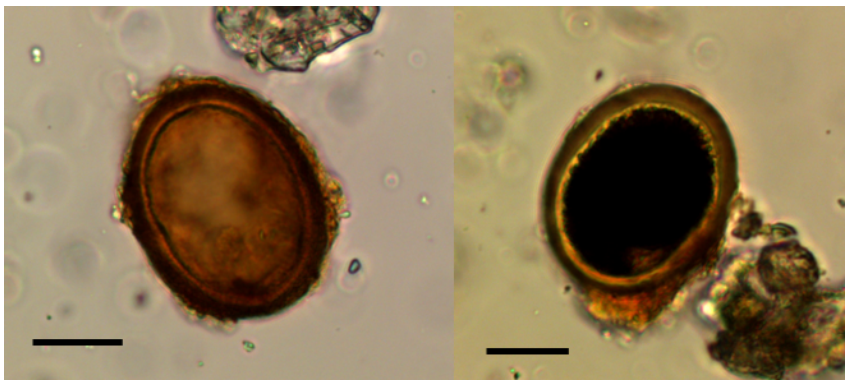

Figure 38: *Ascaris* sp. eggs from pelvic soil of Individual D from Oplontis. Scale bars are 20µm.

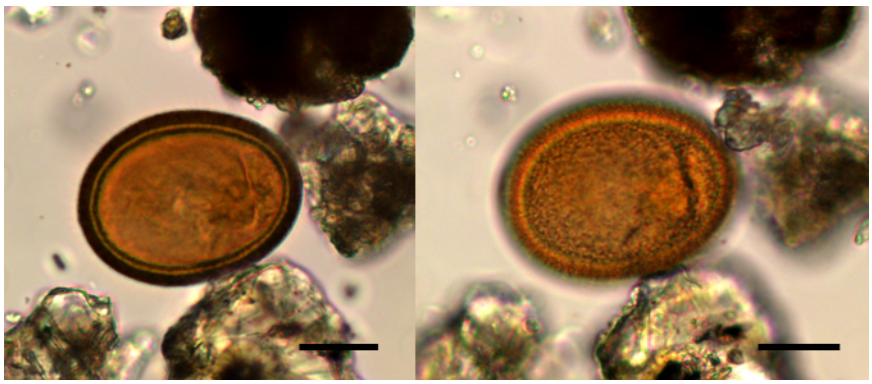

Figure 39: An *Ascaris* sp. egg from Oplontis. The surface structure of the egg is visible in the image on the right. Scale bars are 20µm.

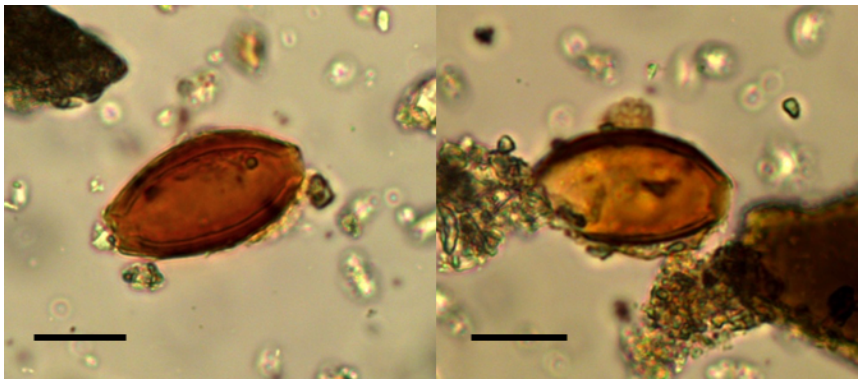

Figure 40: *Trichuris trichiura* eggs from the pelvic soil of Individual D from Oplontis. Scale bars are 20µm.

4.3.7 Vagnari

4.3.7.1 Materials

Vagnari is an Imperial Roman estate in southern Italy, located near the modern-day city of Gravina in Puglia. The site consists of a *vicus* (village) and associated cemetery. During the

expansion of the Roman Republic into southern Italy in the 2nd c. BCE land was seized by the Roman state and local Italian populations were enslaved or employed (Roselaar, 2014). Land seized in this time period became public property of the Roman people or was parcelled out to certain individuals. Some of this public land was later claimed by Roman elite and established as private estates. In the case of the estate at Vagnari, stamped tiles suggest that the estate was owned by the Emperor and individuals working on it may have been enslaved or employed (Small, 2011b). Archaeological support for the hypothesis that workers at Vagnari were slaves comes from the finding of a tile stamped with GRATI CAESARIS (*of Gratus (slave) of Caesar*) (Small, 2011a). Stable isotope and mitochondrial DNA studies on skeletons from the Vagnari cemetery suggest that the majority of people buried at Vagnari were local or immigrated from nearby southern Italy (Emery et al., 2018a; Emery et al., 2018b).

The *vicus* at Vagnari was constructed in the area of a minor Iron Age site, and became the centre of the Imperial Estate around the 1st c. CE. Industries including tile production, iron working, lead processing, and wine production were established (Small, 2011b; Prowse and Carroll, 2017). The area around the estate also likely continued to be used as pastures, as it had been in the Iron Age. These industries would have been established to produce revenue for the Emperor. The excavated areas of the *vicus* so far have revealed a number of buildings. Notably one of the buildings consists of a series of small rooms with beaten earth floors and no hearths or other domestic features, which may have been for housing of slaves or workers, or storage of goods (Carroll, 2014). There are also buildings that appear to be dedicated to specific industries, as well as an area for wine production and a number of kilns around the site. There were a series of drains running underneath the buildings at the *vicus* which were hypothesized to contain waste or water run-off (Figure 41), though the origins of these drains is still unknown. Four sediment samples from the fill of three drains were collected to test for parasites (Table 25), and the presence of faecal waste in these drains. These samples were collected by excavators at the site under the direction of Maureen Carroll.

Table 25: Details of samples collected from drains at the *vicus* at Vagnari.

Drain Number	**Context Number**
5013	5020
5045	5046
5045	5050

5051 5065

Figure 41: Excavated drain from Vagnari *vicus*. Image credit: Maureen Carroll.

The cemetery associated with the *vicus* at Vagnari was in use between the 1st–4th centuries CE. To date around 140 burials have been excavated, with excavations starting in 2002. The most common burial type in the cemetery is *alla cappuccina* burials constructed by placing the body in a shallow pit that is covered with large roofing tiles (*tegulae*) in an inverted 'v' then capped with *imbrices* (Figure 42). Other burials are in pits with no coverings or covered with a layer of flat roofing tiles, and some also contain libation tubes. Most burials are inhumations, only four cremation burials have been excavated so far (none of which were sampled for paleoparasitological analysis) (Prowse and Carroll, 2017). Starting in 2012, soil samples were collected from the pelvis of skeletons for palaeoparasitological analysis. Samples were collected from directly on top of the sacrum and control samples were collected from inside or around the cranium and around the feet where possible. Currently 22 individuals have been sampled and were studied as part of this doctoral research (Table 26). These samples were collected by myself and other excavators at the site under the direction of Tracy Prowse.

Figure 42: Example of an *alla cappuccina* burial from the cemetery at Vagnari (F327). Image Credit: Tracy Prowse.

Table 26: Details of burials from the Vagnari cemetery from which soil samples were collected from the pelvis.

Feature Number	Burial Type	Age	Sex	Samples Collected	Helminth Eggs in Pelvic Soil
F289	*Cappuccina*	15–16 years	Undetermined	Pelvis and control from cranium and feet	-
F290	*Cappuccina*	45+ years	Male	Pelvis and control from cranium and feet	-
F291	*Cappuccina*	Adult	Male	Pelvis and control from cranium and feet	-
F298	*Cappuccina*	Adult	Female	Pelvis and control from midchest	-
F306	*Cappuccina*	Adult	Female	Pelvis and control from cranium and feet	-
F308B	*Cappuccina*	Adult	Male	Pelvis and control from cranium and feet	-
F309	*Cappuccina*	Young Adult	Male	Pelvis and control from cranium and feet	-
F312	*Cappuccina*	Young Adult	Male	Pelvis and control from cranium and feet	-

F313	*Cappuccina*	19–23 years	Female	Pelvis and control from cranium and feet	-
F314C	*Cappuccina*	8–11 years	Undetermined	Pelvis and control from cranium and feet	-
F315	*Cappuccina*	20–27 years	Male	Pelvis and control from cranium and feet	-
F318	*Cappuccina*	Adult	Male	Pelvis and control from cranium and feet	-
F319	*Cappuccina*	Young Adult	Female	Pelvis and control from cranium and feet	-
F320	*Cappuccina*	27–49 years	Female	Pelvis and control from cranium and feet	-
F321	Pit	Young Adult	Male	Pelvis and control from cranium and feet	-
F323	*Cappuccina*	33–58 years	Female	Pelvis and control from feet	-
F324	Flat tile	Adult	Female	Pelvis and control from cranium and feet	-
F327	*Cappuccina*	33–58 years	Male	Pelvis and control from cranium and feet	-
F331	Pit	Adult	Male	Pelvis and control from cranium and feet	-
F333	*Cappuccina*	30–49 years	Female	Pelvis and control from cranium and feet	-
F336	*Cappuccina*	8–12 years	Undetermined	Pelvis and control from cranium and feet	-
F345B	*Cappuccina*	15–16 years	Female	Pelvis and control from cranium and feet	-

Note: Age and sex data were collected by Tracy Prowse and Marissa Ledger.

4.3.7.2 Results

No helminth eggs were found in the subsamples of pelvic soil from the 22 burials sampled from Vagnari. ELISA analysis was not used to test for protozoan parasites in these samples due to the low yield of pelvic soil and high costs of the tests.

Eggs from *Ascaris* sp. were found in subsamples studied from one of the three drains in the *vicus* (village) at Vagnari (Figure 43). These eggs were found in very low concentrations and were poorly preserved. The only sample that had eggs in it was Context 5046 in drain 5045. There were two sediment samples collected from drain 5045 but only this one sample had eggs in it. A total of 5 eggs were found, giving a concentration of 25 eggs/g.

The mean length of the eggs was 62.5 µm (SD 2.4) and the mean width was 48.8 µm (SD 1.6). All eggs were fertile and decorticated.

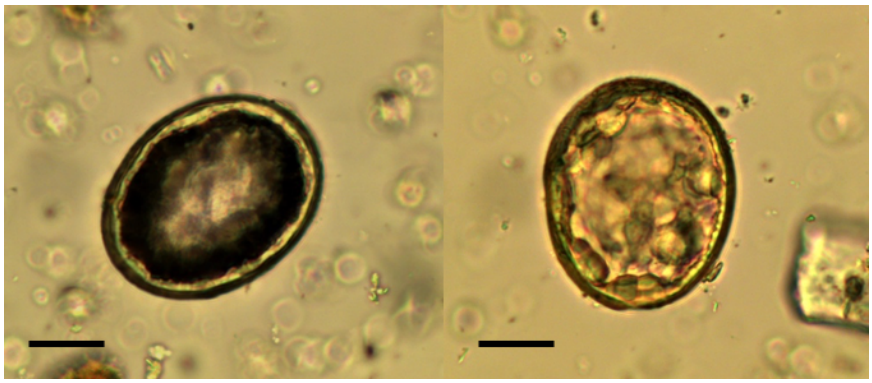

Figure 43: *Ascaris* sp. eggs from Vagnari *vicus* drain 5045 (context 5046). Scale bars are 20µm.

It is not possible to differentiate between *A. lumbricoides* from humans and *A. suum* from pigs and as mentioned earlier these parasites are not necessarily host specific. Therefore, it is possible that these eggs could have been washed into the drains if animal (especially) pig faeces contaminated this area of the *vicus* and were washed into the drains. A latrine has not yet been found on the *vicus* site, however there likely was one somewhere in the area that has not yet been excavated. It is possible that the drains studied were connected to latrines or even a bath complex in other areas of the site, which is how *Ascaris* sp. eggs from human infections could wind up in the drains. The low concentration of eggs in the drain sample suggests that the area was not heavily contaminated with faecal material or few individuals/animals were infected. They may also be reflective of poor preservation at the site. Compared to skeletal remains from Roman period Britain the skeletons at Vagnari are poorly preserved, though there is a range in completeness. This may also play a role in the negative results from the pelvic soil samples.

ELISA analysis was not used to test for protozoan parasites in the pelvic soil or drain samples from Vagnari. Due to the high cost of the tests and the low concentration of eggs in the Vagnari drain, which may indicate a low concentration of faecal material. At the time of writing, other samples were prioritized for testing, however, this sample will be processed on a later run when extra wells are available.

4.3.8 The Vacone Villa

4.3.8.1 Materials

The Roman villa of Vacone is being excavated as part of the Upper Sabina Tiberina Project. The villa is located 55 km north of Rome in the small town of Vacone. The first period of construction of the villa was in the late-Republican period (2nd c. BCE) however, it appears to have been extensively renovated in the early Imperial period (the time of Augustus) (Bloy et al., 2014). This rural villa was extensively decorated with numerous preserved mosaic floors and painted plaster walls (Bloy et al., 2017). The villa consists of numerous rooms for living and production. Areas dedicated to olive oil production have been identified, and agricultural activity also took place around the villa. The villa was abandoned in the early 3rd c. CE after it was damaged, possibly by an earthquake. Some attempts at repair were made but the villa was eventually abandoned. There is then another phase of activity at the site at the time of Lombard expansions into Italy, and several burials have been found dating from the 7th to 8th c. CE. For further discussion of the post-Roman occupation see section 4.4.2 below.

Sediment samples were collected from three areas at the villa that were thought to contain faecal material, either because they were sewage drains or latrines. The first was from the subfloor of Room 33, a potential latrine at the villa (VAC1, 1142, Room 33) (Figure 44). This room was suspected to be a latrine (personal communication) and soil was collected from the floor of the room. Three samples were collected from the room for analysis. The second set of samples came from a *cappuccina* drain that may have carried faecal material and other sewage (VAC1, 1350, *cappuccina*) (Figure 44). Three soil samples were collected from this drain. The third set of samples came from another drain excavated from the site in 2019 (VAC19, drain 931). The drain was sampled in four locations: 1) the exposed drain entrance (context 1514); 2) the first 2m of the drain channel (context 1514.1); 3) 2m down the drain channel to the corner (context 1514.2); and 4) at the corner of the drain (context 1514.3). The samples were obtained from a collaboration with Candace Rice and Erica Rowan. The samples from drain 931 were collected by Erica Rowan who also studied them for archaeobotanical and insect remains. Her results strongly suggested that this was a sewer likely to contain faecal material. Identification of human parasites in these samples could also confirm the suspected purpose of these contexts as part of the sanitation infrastructure of the villa with a primary use to carry faecal material and waste.

Figure 44: *Cappuccina* drain (left) and the possible latrine, Room 33 (right) from the villa at Vacone. Image credit: Upper Sabina Tiberina Project.

Table 27: Description of Roman period samples collected and analysed from the villa at Vacone.

Sample	Context Number	Description of sampling location
Room 33, subfloor	1142	Floor of possible latrine
Cappuccina drain	1350	Drain
Drain 931	1514	Opening of drain
	1514.1	Opening to 2m
	1514.2	2m to corner
	1514.3	corner

4.3.8.2 Results

Similar to Vagnari, the only helminth eggs found in the sediment samples from the villa at Vacone were *Ascaris* sp. In subsample 1 from Room 33 one *Ascaris* sp. egg was found (Figure 45), giving a concentration of 5 eggs/g in the sample. It was suspected during excavation that this room may have held a latrine (personal communication). The egg found

measured 69.4 µm in length and 50.1 µm in width. This fits within the average size range for modern roundworm eggs. The egg was decorticated (missing its outer mamillated coat) and fertile. As the egg was found in a sample taken from the subfloor of the room it is possible it was deposited there during use of the room or even during construction of the floor if it was present in sediment moved from another area of the site.

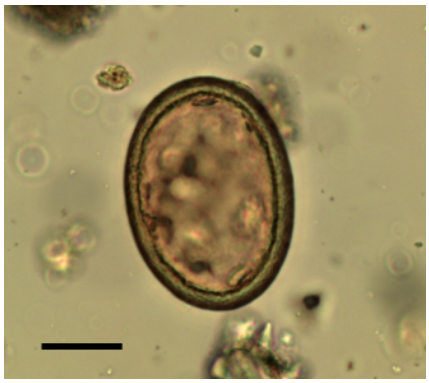

Figure 45: *Ascaris* sp. egg found in Room 33 from the villa at Vacone. Scale bar is 20µm.

No eggs were found in the subsamples from the *cappuccina* drain. This could be because it was used primarily to carry water runoff rather than waste. Alternatively, it is also possible that any faecal material and parasite eggs present in the drain did not preserve. If the drain was still functioning to carry water after the abandonment of the villa it is possible that any eggs present were washed away.

ELISA was not done on the samples from the Vacone villa due to the low concentration of eggs found in the first subsamples studied, which may indicate a low concentration of faecal material in the samples. The samples from drain 931 were obtained close to the date of submission of this thesis so were not tested. The subsamples from drain 931 will be tested at a later date when additional ELISA kits are available.

Ascaris sp. eggs were found in higher concentrations in the samples from drain 931. The egg concentrations in the four samples ranged from 140–620 eggs/g. All eggs were decorticated with the mean length ranging from 59.6–61.6 µm and the mean width ranging from 46.5–46.6 µm in the different samples (Table 28). In addition, one *Trichuris* sp. egg was found in context 1514.3 from drain 931. This is the only whipworm egg found in any sample from the villa at Vacone. This egg was 47.5 µm long and 25.2 µm wide, missing both polar plugs (Figure 46). These results strongly suggest that this drain was used to carry sewage including human faecal material.

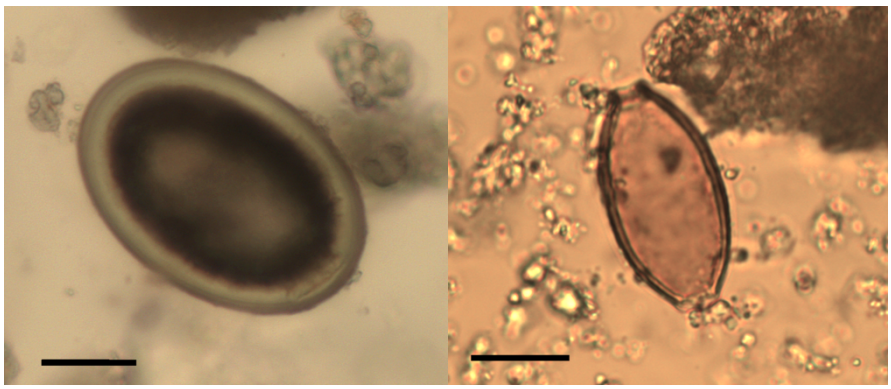

Figure 46: *Ascaris* sp. egg found in drain 931 (context 1514.1) from the villa at Vacone (left). *Trichuris* sp. egg found in drain 931 (context 1514.3) from the villa at Vacone (right). Scale bars are 20µm.

Table 28: Details of helminth eggs found in samples from the villa at Vacone. For each sample, helminth taxa identified are listed as well as egg concentrations in eggs per gram and mean length and width with standard deviations.

Sample	Context	Species	Eggs/g	Mean Length ± SD (*µ*m)	Mean Width ± SD (*µ*m)
Room 33, subfloor	1142	*Ascaris* sp.	5	69.4	50.1
Cappuccina drain	1350	none	0	N/A	N/A
Drain 931	1514	*Ascaris* sp.	620	61.5 ± 3.4	46.5 ± 1.9
	1514.1	*Ascaris* sp.	405	61.5 ± 2.7	46.5 ± 1.5
	1514.2	*Ascaris* sp.	140	59.6 ± 3.9	46.6 ± 2.1
	1514.3	*Ascaris* sp.	185	61.6 ± 3.6	46.5 ± 1.8
		Trichuris sp.	5	47.5	25.2

4.3.9 Viminacium

4.3.9.1 Materials

The results from Viminacium have been included in a manuscript submitted to the American Journal of Archaeology. The description here is adapted from that work (Ledger et al., under review; see Appendix A.5).

The northeastern frontiers of the Roman Empire were partially located in the Balkans and due to their location as bordering kingdoms to the east they had a large military presence

and numerous forts to protect the border. Viminacium was the capital of the Roman province of *Moesia Superior*, and was a major legionary fortress and city in Serbia, the Danube region of the Roman Empire. It was strategically located near the northern frontier of the empire and at the junction of major roads leading in all directions that were important for military movements and trade in the region. It was likely established in the 1st c. AD as a military camp with a legion permanently stationed there, and due to its location along major roads and rich agricultural land, a thriving city grew around it. Viminacium was a base for smaller forts in the region and archaeological excavations have shown it had a number of workshops to supply goods to other forts and cities in the region (Wilkes, 2005). The population of the city has been estimated to be around 40,000 at its peak, and was quite diverse with influences from the Celtic Scordisci who lived there in pre-Roman times, the Dacians on the other side of the Danube, and many travellers from elsewhere in the empire (Golubovi and Mrdić, 2010).

While excavating the city bath complex at Viminacium in 2004 (Figure 47), a public communal latrine was identified. Around its outer circumference there was a channel into which water flowed from the baths to drain faeces from the latrine (Figure 48). Water would have entered the channel in the southwest corner, leaving the room at its northwest corner through the opening in the west wall. The flow of water through the room was enabled by the slope of the floor whose highest angle is registered in the southwest corner, while the lowest is recorded in the northwest corner at the hole in the west wall. Coprolite deposits were discovered on the floor near the northeast corner of the room. Coins found on the floor of the latrine, one of which dates to the second half of the 2nd c. CE, suggest the use of the latrine and the deposition of the coprolites discovered to date from the middle of the 2nd c. to the beginning of the 3rd c. CE (personal communication). A large coprolite found in the drain of the latrine was used to look for preserved parasite eggs. Its surface contour reflects the internal shape of the human colon and rectum (Figure 49). Four separate subsamples were collected from different areas of the coprolite. These samples were obtained through a collaboration with Nataša Šarkić and Saša Redžić.

Figure 47: Photo of Viminacium thermae with latrine and location of coprolite in latrine drain marked by red arrow. Image credit: Nataša Šarkić and Saša Redžić, Viminacium Archaeological Project.

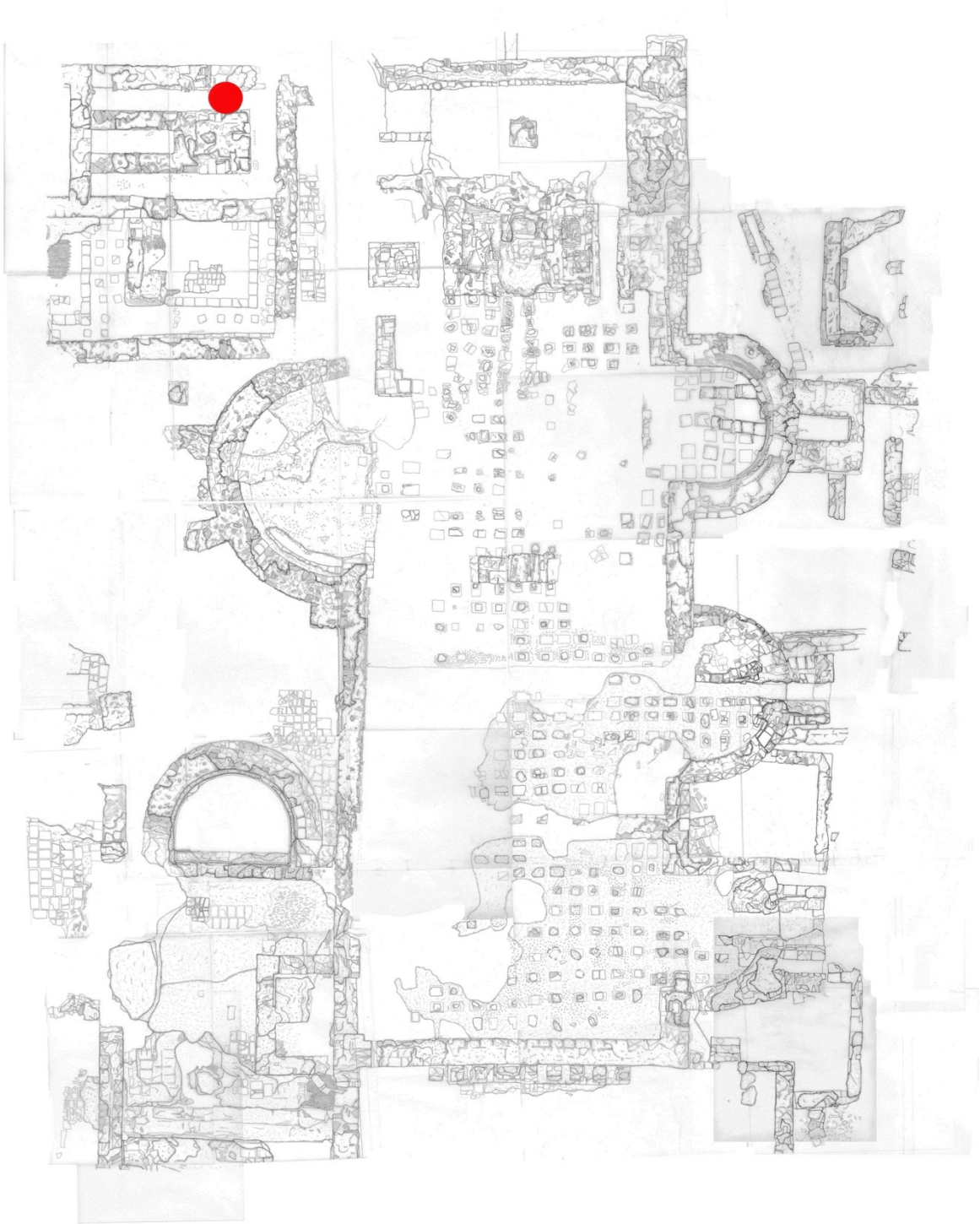

Figure 48: Plan of Viminacium thermae with location of coprolite in latrine marked by red dot in top left corner. Image Credit: Nataša Šarkić and Saša Redžić, Viminacium Archaeological Project.

Figure 49: Coprolite recovered from Viminacium thermae. Its max length is 243 mm, max width 146 mm, and max thickness 60 mm. Image Credit: Nataša Šarkić and Saša Redžić, Viminacium Archaeological Project.

4.3.9.2 Results

In the four subsamples studied from the coprolite recovered from the public latrine at Viminacium, eggs from *Ascaris* sp. and *Trichuris trichiura* were found (Figure 50 and Figure 51). Most roundworm eggs were fertile but two infertile eggs were found, and some had preserved mamillated coats. The mean length of fertile roundworm eggs was 61.4 µm and the mean width of fertile eggs was 48.2 µm (Table 29). The concentration of roundworm eggs in the four different subsamples from the same coprolite ranged from 25–50 eggs/g.

The *Trichuris trichiura* eggs found were also well preserved, some still had polar plugs. The mean length of eggs with both polar plugs was 53.9 µm and without polar plugs was 49.4 µm. The mean width of all eggs was 26.9 µm (Table 29). This fits within standard size ranges for human whipworm both with and without polar plugs, though the mean is within the overlapping range with *T. suis* (Beer, 1976; Garcia, 2016). The concentration of whipworm eggs in the four different subsamples ranged from 65–150 eggs/g. The presence of the eggs in a human coprolite in relatively high concentrations makes them most likely to represent a true human infection with the human-infecting species of *Trichuris,* therefore they have been identified as *T. trichiura*.

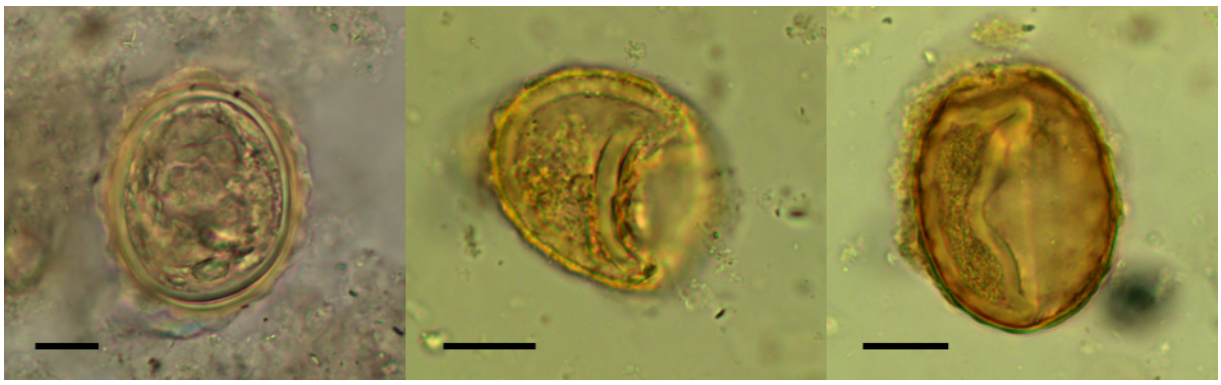

Figure 50: *Ascaris* sp. eggs from Viminacium coprolite. Scale bars are 20µm.

Figure 51: *Trichuris trichiura* eggs from Viminacium coprolite. Scale bars are 20µm.

Table 29: Dimensions of *Ascaris* sp. and *Trichuris trichiura* eggs found in the coprolite from Viminacium.

Species	**Mean Length ± SD (*µ*m)**	**Mean Width ± SD (*µ*m)**
Ascaris sp.		
fertile	61.4 ± 4.9	48.2 ± 4.1
infertile	87.3 ± 0.0	42.8 ± 8.5
Trichuris trichiura	51.5 ± 4.0	26.9 ± 1.7
both polar plugs	53.9 ± 3.1	26.5 ± 1.7
one polar plug	51.8 ± 5.0	27.1 ± 1.7
no polar plugs	49.4 ± 3.4	27.1 ± 1.8

All ELISA tests were negative for the Viminacium coprolite, including technical and biological repeats. On the first test, sample 3 was processed in two different ways due to the fact that trisodium phosphate was not adequate to disaggregate the heavily mineralized coprolite. The first subsample was ground with a mortar and pestle then disaggregated in trisodium phosphate overnight (~19 hours) before sieving. A second subsample was

disaggregated with 1M HCl until all carbonate material was broken up and the sample stopped bubbling when HCl was added dropwise. The sample was then rinsed with distilled water 5 times, centrifuged, and concentrated to a final volume of 2.5 mL. The two variations were done because the stop solution in the ELISA kits is a concentrated acid (HS) and so it is possible that if the sample was not brought back to a nearly neutral pH it would prevent the necessary enzyme reactions in the kits. On the retest the sample was prepared with mortar and pestle and only ran in one column. The absorbance values for these tests can be found in Appendix B.3.

4.3.10 Ephesus

4.3.10.1 Materials

The material from Ephesus has been published and the information here has been taken from that publication: Ledger, M.L. et al. (2018) 'Intestinal parasites from public and private latrines and the harbour canal in Roman period Ephesus, Turkey (1st c. BCE to 6th c. CE)', *Journal of Archaeological Science: Reports*, 21, pp. 289–297 (see Appendix A.4).

The city of Ephesus 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 successors, 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, 2019). 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. These samples and archaeological information about the site were obtained through a collaboration with Friederike Stock, Helmut Schwaiger, Maria Knipping, Helmut Brückner, and Sabine Ladstätter.

The first set of sediment samples analysed from Ephesus were collected from the drain of a public latrine associated with the adjacent Varus bath complex, also called the Baths of Scholastica (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 (Figure 52). All samples date to the 6th century CE, the latest period of use of the latrine.

Figure 52: Photo of the fill of the drain of the public latrine at Ephesus (left); image credit: Friederike Stock. Mineralised material from sewer connected to private latrine at Ephesus (right); image credit: Marissa Ledger. From Ledger et al. (2018).

The second sample was 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 (Figure 52). The seating component of the latrine was destroyed in the 3rd century CE.

4.3.10.2 Results

In the drain from the public latrine at Ephesus eggs of *Ascaris* sp. were found exclusively. Eggs were found in all four samples from different depths in the drain. A total of 20 eggs were found in all samples, all eggs were fertilized and decorticated (Figure 53). In the subsample studied from 0–2cm one egg was found. In the two subsamples studied from 4–6cm deep seven eggs were found (17.5 eggs/g). In the two subsamples studied from 9–11cm deep ten eggs were found (25 eggs/g). Finally, in the subsample studied from 15cm deep two eggs were found (10 eggs/g). The mean length of all eggs was 60.9 µm (SD 3.4) and the mean width was 46.5 µm (SD 2.8).

In the mineralised material from the private latrine at Ephesus eggs of *Trichuris trichiura* were found (Figure 53). A total of 11 eggs were found in the subsample studied (55 eggs/g). All eggs were missing one or both polar plugs. The mean length of the eggs was 50.1 µm (SD 3.6) and the mean width was 26.4 µm (SD 1.2).

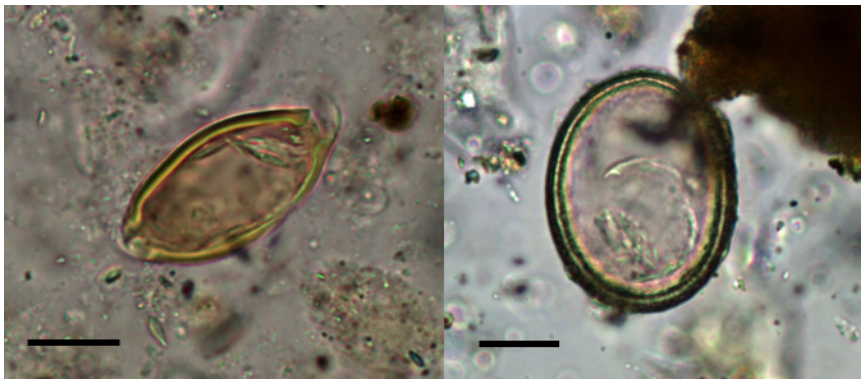

Figure 53: *Trichuris trichiura* egg from Ephesus house latrine and *Ascaris* sp. egg from public latrine drain. Scale bars are 20 µm.

All ELISA results were negative for the samples from the public latrine. The mineralised material from the private latrine was not tested as it needed HCl to be disaggregated and it was thought that this could inhibit the enzymes in the ELISA kits, although it was later shown that this may not be the case (see section 4.3.9). All absorbance values can be found in Appendix B.4.

For an in-depth discussion of microscopy results from the Roman period latrines at Ephesus see Ledger et al., (2018) (Appendix A.4).

4.3.11 Sardis

4.3.11.1 Materials

The results from Sardis have been included in a manuscript submitted to the American Journal of Archaeology. The description here is taken from that work (Ledger et al., under review; see Appendix A.5).

Sardis was a city roughly 150 km northeast of Aphrodisias, and was a Lydian settlement that later became part of the Roman Empire. It flourished during the Imperial period under Roman control, which is when many of its public monuments were built (Greenwalt, 2010). Sardis has a long history as a Lydian city and it is likely that even when it came under Greek control, the mostly indigenous population maintained some of the Lydian cultural heritage while adopting a Greek way of life (Ratté, 2008). In Late Antiquity Sardis became the capital of the new province of Lydia, and flourished in the 4th–5th centuries which is when construction of its monumental synagogue may have begun (Hanfmann et al., 1983). It was a large city, with an estimated population in the Late Roman period around 50,000–100,000 inhabitants (Rautman, 2011). As with other sites in the eastern Mediterranean, Sardis began to decline in the 7th c. CE, with abandonment of certain public spaces, subdivision and repurposing of houses, and abandonment of the Byzantine shops after an earthquake and fire in the early 7th century (Rautman, 2011).

During excavations at Sardis, sediment was collected from two drains within the city. The first drain was connected to a Late Roman house (F55, trench 17.1). This house latrine was located off the courtyard of the house and inhabitants likely had to exit into the courtyard in order to enter the latrine. Based on the building materials and construction, the latrine appears to be a later addition to the house. Similarly, the drain running beneath multiple rooms in the house appears to be an addition in the Late Roman period. The latrine dates from the late 6th c. to early 7th c. CE. The latrine was a small room that had an opening at the back wall, and a drainage system consisting of a pipe that exited to the north underneath room 2 and connected to a larger drain that was sampled (Cahill, 2018:335–337; Cahill, 2019:105–106). This larger drain was also connected to other latrines and would contain faecal material from other parts of the city. Therefore, any parasites found in this drain can give evidence for

infection in the inhabitants of this house and any visitors as well as possibly individuals using latrines in other areas of the city.

Figure 54: Plan of Late Roman house from Sardis with drain running below floors (outlined in red). Location of sediment sample collected from drain attached to latrine in the house is marked by the red dot and the location of the latrine room is marked by the black dot. Image credit: The Archaeological Exploration of Sardis Project.

An additional three samples came from a drain running through the centre of the city (road trench 17.1). This second drain, or series of drains, ran below the synagogue though did not connect to any drainage from the synagogue, but would have carried waste material from elsewhere in the city. There were multiple branches to the drain that are thought to join a larger drain later in their course, samples were collected from two branches that joined together downstream. Similar to the drain near the Late Roman house, this drain dates to the Late Antique period, and was probably used beginning in the 6th c. CE until the 7th c. CE. The drains sampled run beneath a portico along a major street in the city. The area they are connected to is inaccessible as it is under a modern road. On the other side of the modern road is a residential area of the site so it is possible that the drains were connected to houses in this area. Thus, parasite eggs found in the city drain can give wider population-level evidence for parasitic infection in the general population of the city.

Samples from Sardis were collected by the excavators of the site and were obtained through a collaboration with Nicholas Cahill and Erica Rowan. Additional information about the context of the samples was provided by Frances Gallart Marques and John Sigmier.

4.3.11.2 Results

Parasite eggs were present in samples from both the city drains and the drain connected to the house latrine from Sardis. Eggs from *Ascaris* sp. were found in all samples studied; this was the only helminth found in the samples (Figure 55). In the drain connected to the house latrine, *Ascaris* sp. eggs were found in a lower concentration of 55 eggs/g compared to the city drain, where the concentration ranged from 170–305 eggs/g. The mean length of all *Ascaris* sp. eggs was 62.2 µm (SD 4.3) and the mean width was 47.3 µm (SD 2.5). The dimensions of eggs in the different samples can be found in Table 30. All eggs were fertile and decorticated. Due to the lack of distinction between human and pig roundworm, and the apparent low host-specificity of the two species (Betson et al., 2014), I have identified the eggs found as *Ascaris* sp.

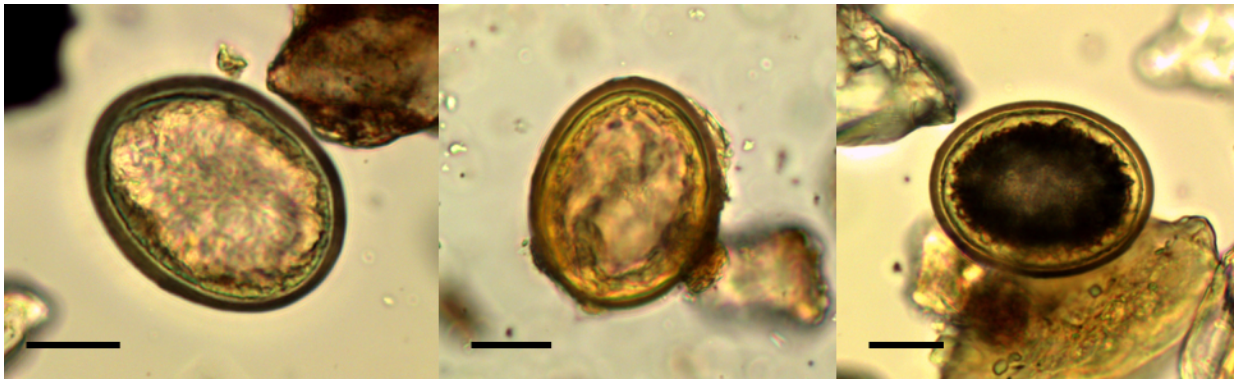

Figure 55: *Ascaris* sp. eggs from Sardis drains. Scale bars are 20 µm.

Table 30: Dimensions and egg counts of *Ascaris* sp. eggs from drains at Sardis.

Sample	Location	Species	Number of Eggs	Eggs/g	Mean Length ± SD (*μ*m)	Mean Width ± SD (*μ*m)
F55, Trench 17.1, Basket 59	House latrine drain	*Ascaris* sp.	11	55	62.9 ± 4.9	46.0 ± 2.1
RT, Trench 17.1, Basket 66 Top	City drain	*Ascaris* sp.	34	170	62.5 ± 4.0	47.5 ± 2.8
RT, Trench 17.1, Basket 66 Bottom	City drain	*Ascaris* sp.	61	305	61.9 ± 3.8	46.5 ± 2.1
RT, Trench 17.1, Basket 67	City drain	*Ascaris* sp.	41	205	62.2 ± 5.1	48.5 ± 2.4

All three ELISA tests were negative for protozoan parasites in the samples from Sardis. Absorbance values can be found in Appendix B.5.

4.4 Post-Roman Sites

4.4.1 Hatherdene Close Cemetery

4.4.1.1 Materials

The cemetery at Hatherdene Close, Cherry Hinton, Cambridge was excavated by the Oxford Archaeological Unit in 2016/2017. It was found to contain remains of individuals who lived during the Anglo-Saxon period (5th–6th c. CE). Samples were collected by the Oxford Archaeological Unit and obtained through a collaboration with Richard Mortimer and Natasha Dodwell.

A total of 92 individuals were studied from the cemetery (Table 31). Soil samples from the anterior surface of the sacrum and the sacral foramina of each individual were analysed for preserved parasite eggs and collected following published standards (Mitchell, 2017a). Control samples from the cranium and feet of each individual were also collected. These samples were collected by the Oxford Archaeological Unit and then analysed in the Ancient Parasites lab at the University of Cambridge, UK.

Table 31: Skeletons from Hatherdene Close from which soil was collected from the pelvis for analysis.

Skeleton Number	Samples Collected	Helminth Eggs in Pelvic Soil
201	Pelvis and controls from cranium and feet	-
205	Pelvis and controls from cranium and feet	-
209	Pelvis and controls from cranium and feet	-
220	Pelvis and control from feet	-
221	Pelvis and control from cranium	-
224	Pelvis and controls from cranium and feet	-
225	Pelvis and controls from cranium and feet	-
226	Pelvis and controls from cranium and feet	-
228	Pelvis and controls from cranium and feet	-
241	Pelvis and controls from cranium and feet	-
246	Pelvis and control cranium	-
249	Pelvis and controls from cranium and feet	-
256	Pelvis and controls from cranium and feet	-
259	Pelvis and controls from cranium and feet	-
274	Pelvis and controls from cranium and feet	-
285	Pelvis and controls from cranium and feet	-
294	Pelvis and control from cranium	-
297	Pelvis and controls from cranium and feet	-
300	Pelvis and controls from cranium and feet	-
323	Pelvis and controls from cranium and feet	-
325	Pelvis and controls from cranium and feet	-
353	Pelvis and controls from cranium and feet	*Ascaris* sp.
356	Pelvis and controls from cranium and feet	-
366	Pelvis and controls from cranium and feet	-
373	Pelvis and controls from cranium and feet	-
376	Pelvis and control from cranium	-
417	Pelvis and controls from cranium and feet	-
422	Pelvis and controls from cranium and feet	-

440	Pelvis and controls from cranium and feet	-
443	Pelvis and controls from cranium and feet	-
468	Pelvis and control from feet	-
493	Pelvis and controls from cranium and feet	-
505	Pelvis and controls from cranium and feet	-
506	Pelvis and controls from cranium and feet	-
526	Pelvis and control from cranium	-
554	Pelvis and controls from cranium and feet	-
557	Pelvis and controls from cranium and feet	-
559	Pelvis and controls from cranium and feet	-
603	Pelvis and controls from cranium and feet	-
611	Pelvis and controls from cranium and feet	-
634	Pelvis and controls from cranium and feet	-
640	Pelvis and controls from cranium and feet	-
691	Pelvis and controls from cranium and feet	-
705	Pelvis and controls from cranium and feet	-
716	Pelvis and controls from cranium and feet	-
737	Pelvis and controls from cranium and feet	-
767	Pelvis and controls from cranium and feet	-
856	Pelvis and controls from cranium and feet	-
956	Pelvis and controls from cranium and feet	-
964	Pelvis and controls from cranium and feet	-
967	Pelvis and controls from cranium and feet	-
976	Pelvis and controls from cranium and feet	-
999	Pelvis and controls from cranium and feet	-
1016	Pelvis and controls from cranium and feet	-
1036	Pelvis and controls from cranium and feet	-
1051	Pelvis and controls from cranium and feet	-
1056	Pelvis and controls from cranium and feet	-
1059	Pelvis and controls from cranium and feet	-
1070	Pelvis and controls from cranium and feet	-

1073	Pelvis and controls from cranium and feet	-
1092	Pelvis and controls from cranium and feet	-
1095	Pelvis and controls from cranium and feet	-
1098	Pelvis and controls from cranium and feet	-
1116	Pelvis and controls from cranium and feet	-
1119	Pelvis and controls from cranium and feet	-
1127	Pelvis and controls from cranium and feet	-
1134	Pelvis and controls from cranium and feet	-
1138	Pelvis and controls from cranium and feet	-
1140	Pelvis and controls from cranium and feet	-
1148	Pelvis and controls from cranium and feet	-
1149	Pelvis and controls from cranium and feet	*Trichuris trichiura*
1151	Pelvis and controls from cranium and feet	-
1159	Pelvis and controls from cranium and feet	-
1164	Pelvis and controls from cranium and feet	-
1165	Pelvis and controls from cranium and feet	-
1168	Pelvis and controls from cranium and feet	-
1178	Pelvis and controls from cranium and feet	*Dicrocoelium dendriticum*
1182	Pelvis and controls from cranium and feet	-
1186	Pelvis and controls from cranium and feet	-
1202	Pelvis and controls from cranium and feet	-
1229	Pelvis and controls from cranium and feet	-
1261	Pelvis and controls from cranium and feet	-
1266	Pelvis and controls from cranium and feet	-
1272	Pelvis and controls from cranium and feet	-
1275	Pelvis and controls from cranium and feet	-
1293	Pelvis and controls from cranium and feet	-
1300	Pelvis and controls from cranium and feet	-
1303	Pelvis and controls from cranium and feet	-
1311	Pelvis and controls from cranium and feet	-
1314	Pelvis and controls from cranium and feet	-

1319	Pelvis and controls from cranium and feet	-
1326	Pelvis and controls from cranium and feet	-

4.4.1.2 Results

A single parasite egg was found in the pelvic soil from three different individuals studied from Hatherdene Close, this was out of the total of 92 individuals studied. Skeleton 353 had one decorticated roundworm (*Ascaris* sp.) egg in the subsample studied. Skeleton 1149 had one whipworm (*Trichuris trichiura*) egg in the subsample studied, and skeleton 1178 had one egg from the lancet liver fluke (*Dicrocoelium dendriticum*). The subsamples studied from both the head and feet of these three individuals did not contain any parasite eggs. None of the other individuals studied had any parasite eggs in the soil collected from their pelvis.

Due to the presence of only a single egg in the 0.2 g subsamples studied it is difficult to determine if these eggs represent true infections. Though these three individuals did not have any corresponding eggs in the control samples from the cranium or feet, with such a low concentration of eggs in the pelvic soil their presence needs to be interpreted with caution. It is possible that the soil in the cemetery was contaminated with parasite eggs and these could have found their way into the burial contexts in very low levels. Furthermore, it is possible that eggs percolated down from higher levels in the soil. Eggs have been shown to percolate through soil over time to be displaced from their original deposition, however this process is quicker and most problematic in particularly rainy and sandy environments and this is the reason for taking control samples (Camacho et al., 2016).

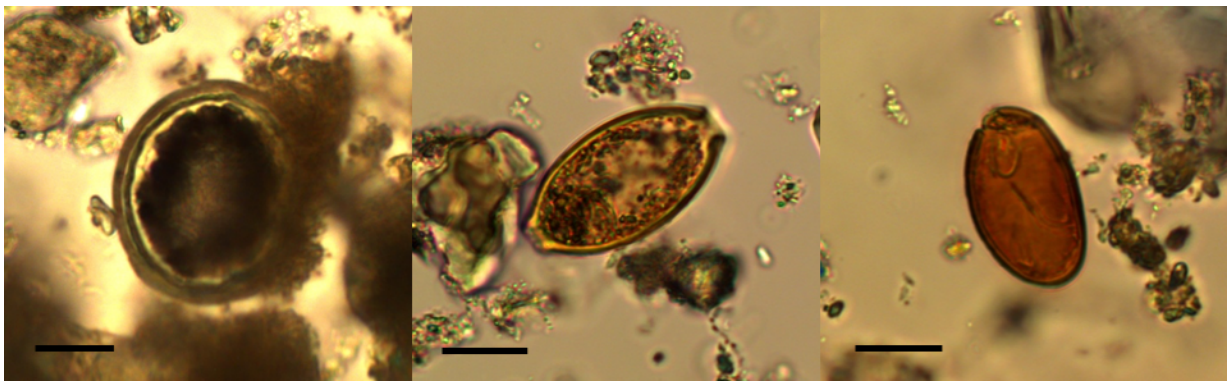

Figure 56: Helminth eggs found in pelvic soil from Hatherdene Close. *Ascaris* sp. egg from skeleton 353 (left); *Trichuris trichiura* egg from skeleton 1149 (centre); and *Dicrocoelium dendriticum* egg from skeleton 1178 (right). Scale bars are 20 µm.

ELISA analysis was not used to test for protozoan parasites in these samples due to the low yield of pelvic soil and high cost of the tests.

4.4.2 The Vacone Villa (Lombard Period Burials)

4.4.2.1 Materials

As mentioned in section 4.3.7.2, after initial abandonment of the Roman villa at Vacone there was renewed activity at the site in the Migration period during the Lombard occupation of Italy. Though there is no evidence that the villa was occupied or used as a dwelling in this later phase there are a series of burials around the villa that date between then 7th–8th c. CE. Pelvic soil was collected from the burials as it could provide useful information about what parasites continued to be present in the area after Roman occupation, similarly it is possible that Lombard individuals carried parasites with them from northern Europe and we may see a change in diversity in this later time period. Primary osteological analysis of these individuals and collection of pelvic soil was done by Devin Ward. Currently four burials have been sampled from the site and excavations are ongoing to collect more (Table 32).

Table 32: Details of burials from Vacone from which soil samples were collected from the pelvis for analysis.

VAC No.	Grave Number	14C Date	Age	Sex	Samples Collected	Helminth Eggs in Pelvic Soil
1	1	660–770 cal CE	3–4 years	Undetermined	Pelvis and controls from cranium and feet	-
1	3	608–684 cal CE	30–42 years	Male	Pelvis and control from cranium	-
1	5	650–750 cal CE	40–50 years	Male	Pelvis	*Ascaris* sp.
2	1	Not dated	20–23 years	Male	Pelvis and controls from cranium and feet	*Ascaris* sp.

Note: Age and sex estimations were done by Devin Ward.

4.4.2.2 Results

Two of the four Lombard period individuals studied had roundworm eggs in the soil in their pelvis, these were VAC1, Grave 5, Individual 1 and VAC2, Grave 1, Individual 1 (Figure 57). Both of these individuals are adult males. A total of 6 eggs were found in the 0.2 g subsample studied in both individuals giving an egg concentration of 30 eggs per gram. For VAC1, Grave 5, Individual 1, the mean length of eggs was 62.3 µm (SD 6.3 µm) and the mean width was 45.9 µm (SD 2.4 µm). For VAC2 Grave 1 Individual 1, the mean length was 59.9 µm (SD 2.1 µm) and the mean width was 45.1 µm (SD 5.7 µm). All eggs recovered from the

pelvic soil were decorticated (missing their mamillated coat or outermost layer) which is common for eggs recovered from archaeological samples. All eggs were fertile, infertile *Ascaris* sp. eggs are larger than fertile and more elongated. There were no control samples to be studied from VAC1 Grave 5 Individual 1. The control sample from the feet of VAC2, Grave 1, Individual 1 contained a single roundworm egg in the 0.2 g subsample. The sample from the cranium of this individual contained three roundworm eggs (15 eggs per gram). Though eggs were found in higher concentrations in the pelvis of this individual compared to the control samples, it is possible that there was general contamination of the site with roundworm eggs and these were present in the burial soil. Thus, it is unclear if the eggs found in the pelvis represent true infection of the individual or contamination of the surrounding soil.

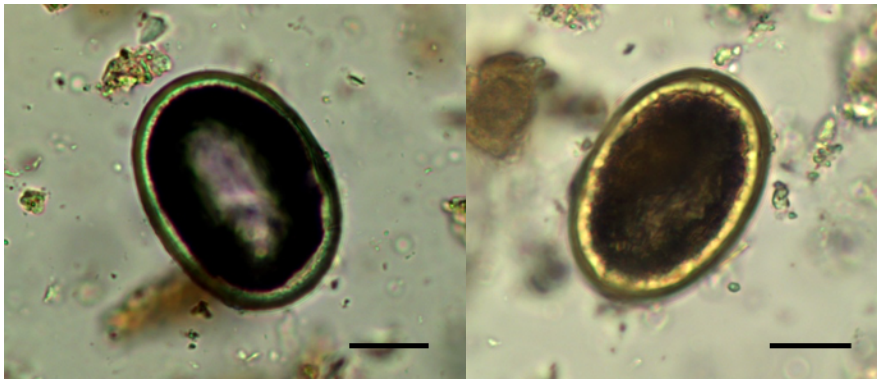

Figure 57: *Ascaris* sp. eggs from Vacone Lombard period pelvic soil. Scale bars are 20 µm.

4.5 Summary of Results for All Sites

The most common species identified in all Roman sites studied was *Ascaris* sp., which was found in nine of the twelve Roman period sites studied. This fits with the overall pattern seen in Roman sites studied by other authors so far. In addition, *Trichuris* sp. was found in five of the twelve sites. The Roman period site with the highest taxonomic diversity was Arlon in Belgium where *Ascaris* sp., *Capillaria* sp., *Dicrocoelium dendriticum*, and *Trichuris trichiura* were all found together in sediment from a latrine.

In the pre-Roman sites, the samples from the Late Bronze Age settlement of Must Farm, UK had the highest taxonomic diversity of any site studied where five taxa were found in the sediment and coprolite samples. In the Neolithic samples from Çatalhöyük and Boncuklu in Turkey no helminth eggs were found in the pelvic soil samples. Similarly, no eggs were found in the pelvic soil of the Griffin Warrior from Mycenean Greece.

All of the post-Roman samples studied for this dissertation were pelvic soil. Few parasites were found in these samples. From the site of Hatherdene Close a single egg was found in the pelvic soil of three individuals, these include an *Ascaris* sp. egg, a *Dicrocoelium dendriticum* egg, and an egg from *Trichuris trichiura*. From the Lombard period burials at the villa of Vacone *Ascaris* sp. eggs were found in two of four individuals. However, this result should be interpreted with caution because *Ascaris* sp. eggs were also found in the control samples from one of the two individuals.

These results will be discussed in detail in the following section. First, I will place these results within regional categories in the Roman Empire to explore how parasite diversity was different in various regions and how cultural variations, sociocultural factors, and local ecology may have influenced parasite diversity in these different regions. Then in Chapter 6 the results will be considered in combination with previously published work from Pre-Roman, Roman, and Medieval sites to analyse temporal changes in parasitic infection in the Roman period.

Table 33: Summary of helminths found at each site studied.

Site	Date	Sample Type	Helminths
Pre-Roman			
Must Farm	9th c. BCE	Sediment Samples	*Capillaria* sp., *Diphyllobothrium* sp., *Dioctophyma renale*, *Trichuris* sp.
		Coprolites	*Capillaria* sp., *Diphyllobothrium* sp., *Dioctophyma renale*, *Echinostoma* sp., *Trichuris* sp.
Çatalhöyük	7100–6300 BCE	Pelvic Soil	None
Boncuklu	8300–7800 BCE	Pelvic Soil	None
Griffin Warrior	1450–1400 BCE	Pelvic Soil	None
Roman			
West Smithfield, London	Roman	Pelvic Soil	*Ascaris* sp.
Winterborne, Dorset	Roman	Pelvic Soil	None
Lauriacum	3rd–4th c. CE	Pelvic Soil	None
Arlon	250–280 CE	Latrine	*Ascaris* sp., *Capillaria* sp., *Dicrocoelium dendriticum*, *Trichuris trichiura*
Paphos	Hellenistic/Roman	Pelvic Soil	*Ascaris* sp.
Oplontis	79 CE	Pelvic Soil	*Ascaris* sp., *Trichuris trichiura*
Vagnari	2nd–4th c. CE	Pelvic Soil	None
		Drains	*Ascaris* sp.
Vacone Villa (Roman period)	Roman	Drains and Latrine	*Ascaris* sp., *Trichuris* sp.
Viminacium	2nd–3rd c. CE	Coprolite	*Ascaris* sp., *Trichuris trichiura*
Ephesus	3rd–6th c. CE	Latrines	*Ascaris* sp., *Trichuris trichiura*
Sardis	6th–7th c. CE	Drains	*Ascaris* sp.

Post-Roman			
Hatherdene Close	5th–6th c. CE	Pelvic Soil	*Ascaris* sp., *Dicrocoelium dendriticum* *Trichuris trichiura*
Vacone Villa (Lombard period)	608–770 CE	Pelvic Soil	*Ascaris* sp.

5 Regional Case Studies

In the following chapter, I will discuss the results from the various sites studied within a regional framework. These regions were chosen to broadly match regional categories of the Roman period, whether that be a specific province or group of provinces. It is important to note that the interconnectedness, mobility of individuals, and trade of both ideas and material items between parts of the empire means that these categories could be redrawn and grouped in numerous ways. I have chosen regional categories that allow for consideration of different aspects of ecology and sociocultural contexts while remaining aware of Roman definitions of territory. Similarly, this is by no means the way that individuals may have chosen to be grouped in the past and thus the same for the diseases they carried. These can be thought of as micro-communities. Within a defined set of individuals there may be multiple other ways that people choose to organize themselves, such as by ethnicity, religion, age, status, or occupation, and all of these groupings may impact sociocultural actions. Thus, by focusing on regional groupings the intricacies of these categories are lost.

Though many discussions exist of how culture changed in newly conquered regions of the empire, we also know that the existing practices in many regions were persistent and strongly influenced the daily life of people living within the borders of the empire (Woolf, 1997; Braund, 1998: 22; Huskinson, 2009; Garnsey and Saller, 2015). Thus we would expect that there is not one model of infectious diseases in the Roman period but that the diseases of those living within the empire also varied, and some of these variations are likely regional.

The concept of Romanization, heavily used and debated in the discussion of Roman culture, brings to light the diversity of culture that existed and changed within the centuries of Imperial rule. While the concept of Romanization has been used to describe the process of newly conquered regions becoming more ‘Roman’ in their way of life, a definition often based on cultural practice in the Mediterranean and especially Italy, it also exemplifies the existing variations that must have existed around the empire before the cultural changes that occurred in the Imperial period. Arguments against the use of the term have emphasized the lack of cultural unity within the empire and the lack of a standard Roman culture (Woolf, 1998: 7; Mattingly, 2004). Further to this, it has been argued that cultural change was not simply a mixing of cultural traits from the Mediterranean with existing cultures in new provincial territories, but rather as the empire expanded to new regions the characteristics of Roman cultural itself continuously changed (Woolf, 1997). Keay and Terrenato (2001) propose a redefinition Romanization as the formation of a composite Roman culture made up of a constellation of ideas from various areas which is locally variable with various levels of

acculturation and cultural resistance. Others have favoured to view these processes of cultural change within the framework of globalization theory or world-systems analysis (Hingley, 2005; Pitts, 2008). No matter what theory or framework we accept these discussions highlight the cultural variability that existed within the empire.

At its greatest extent the Roman Empire had territory in three continents, including much of Europe, North Africa, and western regions of the Near East. Estimates of population size in the Roman period are heavily debated and fraught with error (Bagnall and Frier, 1994; Frier, 2000; Scheidel, 2009) however, if we accept a conservative estimate of the population being 50 million in the Roman Empire in the 1st c. CE, with nearly 6 million being defined as Roman citizens, that leaves 46 million not technically defined as Roman in political terms. Most of these citizens were concentrated in Rome, Italy, and the Mediterranean. This is a point that Richard Hingley (2005) uses to show that identity and culture in the Roman Empire must be diverse across the Empire. A large percentage of the population were not defined as Roman citizens, in general these individuals would include slaves and local populations from the provinces. Further to this, extensive trade networks both within and with regions outside the empire were established during this time which likely impacted disease transmission, dietary choices, and transmission of knowledge such as medical practices. In addition, these trade networks allowed for introduction of new foodstuffs and spread of culinary practices, for example the spread of the popular Roman condiment *garum*. For these reasons it is expected that there are also variations in parasite transmission in different regions of the empire. So far there have been few studies directly comparing disease in different regions of the empire, though these few have shown variations in health and disease regionally (Gowland and Redfern 2010; Scheidel 2012; Mays et al. 2018).

In the following sections we will explore variations in parasite diversity in four regions of the Empire: 1) *Italia;* 2) *Asia* and the eastern Mediterranean more broadly; 3) the frontier provinces; and 4) *Britannia*. These regions were chosen as the newly studied sites fit into these categories and provide some of the first evidence for human parasites in these regions or add to previous evidence collected by other researchers.

5.1 *Italia*

5.1.1 Previous Work

As the epicentre of the Roman Empire, Italy, home to the capital city Rome, is where we shall start. This research has increased the amount of evidence for parasitic infection in Roman Italy as prior to this work only three sites had been studied, namely Rome, Pompeii, and

Portus. The finding of parasite remains at Vagnari, Vacone, and Oplontis thus doubles the number of sites studied in Roman Italy. From the previous parasitological studies done in Italy we have evidence for whipworm, *Entamoeba histolytica*, roundworm, and *Fasciola* liver fluke in Roman contexts (Table 8). Sewer samples from one of the best known cities in Roman Italy, Pompeii, contained eggs from *Trichuris trichiura* (Heirbaut et al., 2011). Similarly a sample from a latrine of unknown location in the city of Rome tested positive for *Entamoeba histolytica* using ELISA, however no evidence or record of analysis for helminth eggs using microscopy was reported (Le Bailly and Bouchet, 2015). Finally sediment cores, dated between 90–245 CE, taken from Portus, the main port for the city of Rome located north of Ostia, contained eggs of *Ascaris* sp., *Trichuris* sp., and *Fasciola* sp. which could be from human or animal activity in the area (Dufour, 2015). Based on parasitological evidence from elsewhere in the Roman Empire and in particular in the Mediterranean, it seems likely that the presence of *Ascaris* and *Trichuris* in the core samples from the port may be a result of drainage from buildings around Portus itself, which likely contained human faecal material, and also its connection to the Tiber River where large amounts of sewage were dumped from the city of Rome (35km away) and any communities between Rome and Portus (Gowers, 1995; Keay, 2012; Keay et al., 2014; Taylor, 2015).

5.1.2 New Results

The results of this doctoral research are very much in line with this previous work. In the samples studied from Italy I found solely soil-transmitted helminths. First, in the pelvic soil of individuals who died in Villa B at Oplontis, one individual was infected with roundworm and whipworm and another with only roundworm. While none of the pelvic soil samples from the Imperial Roman cemetery at Vagnari was positive, samples from a drain of the associated *vicus* contained roundworm eggs. Finally, at the Villa of Vacone a single roundworm egg was found in a sample from the subfloor of a room that was thought to be a latrine and in higher concentrations in one drain. The presence of roundworm in the possible latrine does not offer convincing evidence to support or refute the use of this room as a latrine. The higher concentrations of roundworm eggs in the Roman drains suggest that they were carrying faecal material and individuals at the site were likely infected. In addition, one whipworm egg was found in a drain at the villa of Vacone. The egg concentrations in most of these samples were relatively low, especially at Vagnari. While it is difficult to interpret the meaning of egg concentrations due to the number of factors that can influence them, this may imply poor preservation of helminth eggs in the region or low levels of infection in these communities. When we compare the results from Oplontis to the other two sites, the egg concentrations are

higher in the Oplontis samples compared to the others, despite the fact that the Oplontis samples are pelvic soil samples which usually have lower concentrations compared to drains, as they are a reflection of infection in a single individual rather than multiple. At Oplontis, Individual D had 985 eggs/g of *Ascaris* and 30 eggs/g of *Trichuris trichiura* in the pelvis, and no eggs in soil from the cranium; a level that seems to indicate true infection. The drain at Vagnari had *Ascaris* eggs at a concentration of 25 eggs/g, and at Vacone the subfloor of the possible latrine only had one egg while the drains had higher concentrations of eggs ranging from 140–620 eggs/g. These drastic differences in concentrations may be a result of better preservations of eggs at Oplontis because these skeletons were buried in a closed room at the villa, and possibly the eruption of Vesuvius and covering of the remains in volcanic ash may have provided exceptional conditions for preservation. It is also possible that the individuals at Oplontis generally had higher levels of infection or that the skeletal sample studied happened to contain the individuals with the highest rates of infection. From modern epidemiological studies we know that helminth infections are often overdispersed (Crofton, 1971; Churcher et al., 2005), which means that a small proportion of the population carries the majority of the worm burden. It is possible that at Oplontis we happened to sample some of these individuals.

5.1.3 Sites in Context

We will first explore some of the differences amongst these three sites to put them into context and then consider them more generally as a group within the Roman Empire. Two of the sites studied in Italy were villas, Oplontis and Vacone. While there are many definitions of what villas actually were, the simplest definition is a rural residence (Percival, 2002; Pollard, 2016). While the spectrum of what constituted a villa was broad, the countryside was littered with them. One particular area that was known for its elaborate villas, often described as palatial, was the Bay of Naples. This is where many of Rome's influential elite had villas to host guests and showcase their position in society. Oplontis was one of these opulent villas in the Bay of Naples, that consisted of the villa itself and a commercial area. The skeletons studied were found in the commercial area making it possible that they were typical inhabitants of the villa or people who worked and traded at Oplontis, or a combination of the two. Villas also had a production side in most cases, which could be through agriculture, tile production, olive-pressing or wine production to name a few possibilities (Pollard, 2016). The villa at Vacone, located north of Rome, had areas for olive pressing and ample agricultural land that likely formed its economic base. The hilly landscape may be in part responsible for the lack of urbanisation of this region in antiquity thought it was dotted with villas. The villa

showed evidence of significant wealth in its mosaic floors and painted plaster walls, which may have been generated by olive oil production or agriculture or a reflection of the wealth of the villas owner. Vagnari on the other hand, was a different type of property. It was a rural Imperial Estate or *vicus* whose main purpose was production for the emperor. The individuals working and buried at the estate are largely local southern Italian inhabitants of a lower class (Emery et al., 2018b). As such all three sites can be considered rural which is in contrast to the existing parasite data from urban centres of Rome, Pompeii, and Portus.

The occupants of these different sites would have varied, the owners and visitors to the villas would have represented the elite of Roman Italy, while the workers on the estate of Vagnari were non-elite workers or slaves of the emperor. This means that the samples from Vagnari are likely a reflection of infection of lower class workers on a rural estate. On the other hand the villas were likely occupied by individuals who spent a large portion of their time in large urban centres and may have moved around the empire. However, one must consider that the villas, though owned and visited by Italy's elite would have also had numerous staff and slaves that would have used latrines as well. While the context of the samples studied does not in all cases allow us to determine exactly who was infected it is notable that the taxa causing infections did not vary between these groups. Similarly, previous work from urban contexts such as Pompeii and Rome have shown that parasites transmitted by the direct faecal-oral route were found in urban environments just as I have found at rural villas and a rural Imperial estate.

5.1.4 Soil-Transmitted Helminths and Sanitation

From a more general perspective one of the first questions to ask of these results is why were soil-transmitted helminths the dominant category of helminths found across Roman Italy? The answer to this is not simple but a start is to consider social practices and infrastructure that influenced sanitation and hygiene. The amount of archaeological and historical evidence for sanitation infrastructure, water systems, and waste disposal in large urban centres in Roman Italy such as Rome, Pompeii, and Ostia give ample evidence to describe various routes for the spread of soil-transmitted helminths. Studies on the distribution of latrines within the city of Pompeii have shown that there is one sanitation feature (latrine or downpipe indicating upper level latrine) for every two residential properties (Trusler, 2017), in addition to numerous public latrines (Koloski-Ostrow, 2015:5–10). The idea of privacy during toilet use was not necessarily expected, and having upwards of 20 seats in one latrine with about 60 cm of space per person was not uncommon (Petznek et al., 2011:99–106). While this served the purpose of giving many people a latrine to use, and even making it a potential social venture, modern

studies have shown that sharing latrines may actually be worse for the spread of helminth infections and other faecal-oral pathogens compared to a lack of latrines (Heijnen et al., 2014; Oswald et al., 2017). This is likely due to increased exposure to the faecal material of other people in shared latrines (Emch, 1999). In a Roman public latrine, users would have interacted while using these facilities whether it be just by sitting next to one other, sharing a wash basin, or sharing sponge sticks thought to be used instead of toilet paper (Koloski-Ostrow, 2015: 85). Evidence from cities such as Pompeii have also shown that latrines were located in shops, bars, and possibly even small rooms opening directly off the street, and these were likely used by passers-by when the need arose (Hobson, 2009:55–60; Rowan, 2017).

While private latrines have been found commonly in major cities in Roman Italy, such as at Pompeii where almost every house has a latrine, these also had their pitfalls. The design of private latrines varies across the empire. In general, many private latrines had one or two seats and depending on the city could be connected to main sewers or had cesspits to collect excrement (Hoss et al., 2011:51–55). For those that were connected to sewers, most did not have running water and would have needed to be flushed by pouring water down the latrine. The design of an open cesspit, or a poorly drained latrine connecting to public sewers, would have posed a risk for microscopic parasite eggs to be transferred from the latrine into the living spaces. One potential vector for the movement of eggs around the living environment is flies. Large numbers of flies in Roman living spaces have been suggested (Scobie, 1986) and analysis of latrine and sewer samples has attested to their presence (Knights et al., 1983; Rowan, 2014). Many private latrines were located in or adjacent to kitchens (Pérez et al., 2011:113–118). For these latrines located near kitchens flies may have easily transmitted eggs from the latrine onto food preparation areas or food itself if helminth eggs stuck to their legs as they landed in the latrine and then on other surfaces. The eggs of roundworm in particular are sticky, and are known to adhere to flies to be transmitted around living spaces where they could then be ingested and complete their life cycle causing infection in humans (Hall, 1982). Though most evidence for latrines in Roman Italy comes from these large urban centres, their ubiquity would suggest that smaller rural Roman towns also had latrines and their design was likely similar.

Another aspect of sanitation infrastructure that is often discussed in reference to large cities in Roman Italy is drains and sewers. While drains are typically designed for carrying water and sewers for carrying waste such as human excrement, in the Roman period it is thought that these categories likely overlapped in many instances (Rogers, 2018:40). The famed Cloaca Maxima, Rome's largest drain that ran through the city emptying into the Tiber

river is the prime example of the extensive architectural effort placed into designing drainage networks in Roman cities. It is thought that it was primarily designed to carry excess water from the streets but also would have carried various waste such as excrement, bodies, animal carcasses, and other forms of garbage; thus it is also called a sewer (Koloski-Ostrow, 2015: 66). The design of sewer systems varies from city to city and the best evidence comes from Pompeii, Herculaneum, Rome, and Ostia. As already mentioned Rome has an intricate sewage network consisting of the Cloaca Maxima and many tributaries. Pompeii and Herculaneum were both built on slopes which provided a natural direction of flow for sewage into the sea (Koloski-Ostrow, 2015: 76). Both cities had main sewage networks to drain water from overflowing fountains, storms, and sewers that were connected to some latrines that did not have cesspits. At Pompeii stepping stones across streets attest to the use of the natural flow of water to clean streets and also the potential for standing water in areas of the city. Roman towns may have had open street drains or newer towns may have benefitted from urban planning that included building drainage networks underneath streets from the start (Wilson, 2000b). In more rural cities with smaller populations the need for extensive sewage networks may have been lower. At Vagnari for example, a series of small drains has been identified but it is so far unclear what they were connected to or where they would have emptied.

Though sewers and drains would have greatly contributed to removal of waste material from living spaces they could have also posed a significant risk for contamination of living spaces in times of flooding or backups. For example, regular flooding of the Tiber river is known to have occurred in the Roman period. Aldrete has found historical accounts of 42 floods in the city of Rome from 414 BCE to 398 CE, most of which are written about by Livy and Cassius Dio (Aldrete, 2007:14). When these floods occurred the sewers, most of which did not have traps or backwater valves would have flowed back into the city (Wilson, 2000b), depositing any waste that they carried, such as human and animal excrement. On top of regular flooding of rivers, these sewage channels are thought to have regularly backed up due to accumulation of debris and sediment (Koloski-Ostrow, 2015:65, 79). Historical texts refer to individuals who were paid to clean sewers and empty cesspits. Pliny the Younger describes convicts being charged with cleaning sewers and Diocletian's *Edict on Maximum Prices* described the pay of a *cloacarius*, a person to clean *cloaca* or sewers (Taylor, 2015). How effective these individuals were at stopping backups in large sewer networks is unknown. Sewage backflow into the city during times of flooding or backup could increase the rates of soil-transmitted helminth infection, such as roundworm and whipworm, as helminth eggs could be redeposited in living spaces.

Aqueducts were also an important part of sanitation infrastructure in Roman Italy. The large scale construction of aqueducts in Roman Italy began in the 4th c. BCE and though some of the most impressive examples come from the city of Rome, they spread outside Italy under Augustus to be found across the Mediterranean and in North Africa (Wilson, 2009). Along with wells, cisterns, and channels these water systems functioned to supply clean drinking water to those living in Roman cities. These aqueducts fed water into public fountains which people in the city could use to collect drinking water, if they did not have access to a well or cistern. While it was possible to get permission from the Emperor to connect a private residence to piped water, it is thought that this would have only been accessible to those of higher socioeconomic status (Jansen, 2000). Part of the reason for this is that property owners would have had to pay for this connection. While aqueducts and cisterns can generally bring in water free from pathogens, jugs used to collect water from public fountains or basins may have served as a source of contaminants as has been observed in modern populations without piped water (Khairy et al., 1982; Worrell et al., 2016). Access to piped water has been linked to a decrease in soil-transmitted helminth infections, especially roundworm and whipworm, in many modern communities (Strunz et al., 2014). *Ascaris* and *Trichuris* eggs can remain viable in water for up to a year and *Taenia* for 270 days (Geldreich, 1996; Sengupta et al., 2012).

There are multiple potentially human-infecting species within the genus of *Ascaris.* The two that have eggs that look very similar under the microscope are *Ascaris lumbricoides* and *Ascaris suum*. Classically, *Ascaris lumbricoides* has been identified as the human-infecting species while *Ascaris suum* infects pigs. However, recent genetic work has shown that these two species are very similar genetically and it is debated if they are truly distinct species or the same species with regional variants (Betson et al., 2014). In addition the ability for cross-species transmission is high, in modern settings it has been shown that many human infections in developed countries are caused by *Ascaris suum.* Similarly, the human roundworm (*Ascaris lumbricoides*) can also infect pigs (Nejsum et al., 2012). As mentioned earlier, it is for this reason that I have identified eggs as *Ascaris* sp. because their presence in human contexts, even pelvic soil, does not preclude the possibility that they are *Ascaris suum* eggs acquired from pigs. Pigs were a major source of meat in Roman period and particularly so in Italy (King, 1999). In most cases, especially rurally, pigs would have been kept outside of settlement areas. At Vagnari and in southern Italy more generally there is no archaeological evidence for pig sties, though few pigs may have been kept within settlement areas (MacKinnon, 2011:313). It is possible that faecal material from live pigs kept within settlement areas could contaminate living areas, or that faecal material during slaughtering could lead to the presence of eggs in streets and sewers. Similarly, in agricultural areas pig

faecal material may contaminate crops leading to human infections with *Ascaris suum*. It seems likely that many of the eggs found are from the human-infecting species *A. lumbricoides* due to their presence in human contexts however, for these reasons it remains possible that some eggs are from *A. suum*.

5.1.5 Summary

In summary, the sole finding of soil-transmitted helminths in samples studied from Roman Italy is consistent with previously published palaeoparasitological work. It appears that intestinal parasites reliant on poor sanitation and hygiene for transmission were the major contributors to parasitic disease in Roman Italy. So far there is very little evidence for zoonotic parasites in Roman Italy, while *Ascaris* sp. has some zoonotic potential it can also be transmitted directly between humans without an animal host.

Sanitation systems and latrines have been relatively well studied in Roman Italy compared to other regions, mostly in major cities such as Pompeii and Herculaneum. This information allows us to pinpoint potential routes for spread of faecal-oral parasites such as those relating to the design of latrines and sewer networks, access to latrines, and access to drinking water. There are many other sanitation practices that may contribute to the spread of faecal-oral parasites, some of which will be explored in the following sections.

There does not appear to be any differences in the taxa of parasites found in urban centres such as Rome and Pompeii compared to countryside villas such as Oplontis and Vacone, or compared to rural Imperial estates such as Vagnari. Roundworm has been found in samples from all of these sites and whipworm was found at Pompeii, Oplontis, and Vacone. Thus, the first few sites studied in Roman Italy suggest that there were no differences in the types of infections in rural or urban communities though analysis of additional sites would allow for stronger conclusions.

5.2 *Asia* and the Eastern Mediterranean

The purpose of this section is to combine evidence from sites studied in the eastern Mediterranean to explore variations in infection in the eastern Mediterranean compared to the better studied provinces in northern Europe, namely those in France and Britain. In order to find evidence for intestinal parasitic infection in the eastern Mediterranean during the Roman period, a series of sediment samples were collected and analysed from two sites in modern-day Turkey—Ephesus, and Sardis; as well as one from Cyprus—Paphos. These correspond to the Roman provinces of *Asia* and *Cyprus*, respectively. These results are discussed in conjunction with the recently published work from our lab on the Roman site of Sagalassos

(Williams et al., 2017). A large part of this work has been written up in a manuscript submitted to the American Journal of Archaeology and is adapted from that manuscript (see Appendix A.5).

5.2.1 New Results

Helminth eggs were found in all samples studied from Ephesus, Sardis, and Paphos. In sediment samples from the drain of a public latrine in the Baths of Scholastica at Ephesus roundworm eggs were found (17 eggs/g), and whipworm eggs were found in a private house latrine from Ephesus (55 eggs/g). In the drain samples from Sardis only roundworm eggs were found, in lower concentrations in the drain connected to the house latrine (55 eggs/g) compared to the city drain (170–305 eggs/g). No other taxa were found in any of the samples studied. From the pelvic soil studied from Paphos in Cyprus, roundworm eggs were found in the pelvis of one individual. When we combine these results with those from our previously published work on samples from Roman period Sagalassos, another city in Anatolia, we can get a better sense of the parasite taxa found in the eastern Mediterranean. From a public latrine associated with a bath complex at Sagalassos eggs of roundworm (10–45 eggs/g) and *Giardia duodenalis* was found (Williams et al., 2017). The ubiquity of roundworm and whipworm in the eastern Mediterranean is becoming apparent as more sites are studied in the region. To add to this pattern, roundworm and whipworm were also the only parasites found in Roman period samples from Kea in Greece (Anastasiou et al., 2018).

5.2.2 Previous Work

The majority of palaeoparasitological studies performed at Roman period sites have taken place in northern Europe (see 2.3.6). From the Roman provinces of Belgica, Germania Inferior, and Germania Superior, which cover parts of modern day Belgium, Germany, Netherlands and Switzerland; beef/pork tapeworm, *Capillaria* worm, *Entamoeba histolytica*, *Fasciola* liver fluke, lancet liver fluke, roundworm, and whipworm have all been found. From the province of *Britannia,* beef/pork tapeworm, *Fasciola* liver fluke, fish tapeworm, lancet liver fluke, roundworm, and whipworm have been found. At one site, Carnuntum, in the province of *Pannonia Superior* (Austria) roundworm was found. At one site, Künzing, in the province of *Raetia*, whipworm was found. From the provinces of *Aquitania* and *Lugdunensis,* which cover most of modern-day France, beef/pork tapeworm, *Entamoeba histolytica*, fish tapeworm, lancet liver fluke, roundworm, and whipworm have all been found.

Moving to the Mediterranean region, in the province of *Narbonensis*, in southern France, there is evidence for *Entamoeba* which can cause dysentery, *Fasciola* liver fluke, fish

tapeworm, and whipworm (Harter-Lailheugue, 2006; Le Bailly and Bouchet, 2006). In the central and eastern Mediterranean region, Roman sites have been studied from Greece, Israel, Italy and now Turkey. A similar species diversity to northern Europe has been found in Israel (province of *Iudaea*) where evidence exists for beef/pork tapeworm, fish tapeworm, lancet liver fluke, pinworm, roundworm, and whipworm (Harter, 2003; Zias et al., 2006). In Egypt, mummified remains have given evidence for pinworm and beef/pork tapeworm (Horne, 2002, Le Bailly et al., 2010). While in Greece, Italy and Turkey so far there is only evidence for *Giardia duodenalis*, roundworm, and whipworm (Heirbaut et al., 2011; Le Bailly and Bouchet, 2015; Williams et al., 2017; Anastasiou et al., 2018; Ledger et al., 2018.), the same taxa found at the sites analysed in this study. There is no evidence for the zoonotic parasites beef/pork tapeworms, fish tapeworm, and liver flukes that have been found in other regions.

5.2.3 Predominance of Sanitation Related Parasites

Since they are all transmitted by accidental ingestion of faecal material, usually through contaminated food or water, the presence of roundworm, whipworm, and *Giardia duodenalis* in Roman cities in *Asia* and *Cyprus* gives an indication of sanitation and hygiene levels in cities in these provinces. The evidence for roundworm and whipworm across the eastern Mediterranean suggests that while construction of public and private latrines took place in towns throughout the Roman Empire, and were already present in many Greek cities in the east (Gräzer et al., 2011:29–39), these did not stop the transmission of gastrointestinal diseases reliant on the faecal-oral route as one might expect. At Ephesus, for example, eighteen latrines have been identified with the majority having multiple seats (Jansen, 2006). Though toilet use has been linked to a decrease in faecal-oral parasite transmission in modern communities, their presence alone cannot stop transmission and there are various aspects of their design, maintenance, and use as well as other sociocultural practices around hygiene that may undermine simply their presence.

The purpose of sewers and latrines in Roman cities was not aimed at stopping the transmission of pathogens as they are today, as the concept of micro-organisms was not yet understood at that time. Rather, it is more likely that sewers and latrines were built to remove what was viewed as unclean smells and behaviours from public view (Koloski-Ostrow, 2015: 66). The Romans are well known for building large multi-seat latrines across the empire, and these are particularly prominent in bath complexes in the Mediterranean. It is in Greek cities in the Mediterranean that some of the first evidence for multi-seat latrines is found, such as that at Amorgos, Delos, and Thera (Antoniou and Angelakis, 2015:62–64). It appears that these sanitation technologies were then widely adapted by the Romans who built latrines that

could seat even more people at one time. Indeed a majority of the evidence for intestinal parasites from the Eastern Mediterranean comes from these multi-seat latrines such as that at Ephesus and Sagalassos. Although we were not able to collect samples from multi-seat latrines at Sardis, the city had them as well (Yegül, 1986: 22).

There are of course a number of other places where one could come into contact with faecal material in a Roman city, aside from just in the latrines. Text painted and carved on walls and monuments in Roman cities in the Mediterranean give us some indication that not everyone in these cities used latrines. The numerous warnings against defecating in public places such as on graves, beside houses, and in other public places (Wilson, 2000a:310–311) indicate that this was something that happened frequently enough to be an annoyance. There is also evidence for the use of chamber pots in Roman cities, especially by the elite, and these would have been regularly emptied by slaves either into latrines within the house or somewhere acceptable outside the home (Hobson, 2009: 69). It has even been suggested that the elite may have preferred to use chamber pots at home and latrines located on the main floor of residences were primarily for slaves or freedmen working in the house (Trusler and Hobson, 2017). For residences with no latrines, faecal material would have needed to be collected and dumped into latrines, open sewers, the street, or on dung heaps (Taylor, 2015). Cesspits would have required regular emptying and sewers cleaned, which would have been a job for slaves or *stercorarii* (individuals whose job it was to empty cesspits and collect manure). A similar job seems to have existed in Hellenistic cities (Owens, 1983) and thus likely persisted in these Roman period cities in the Eastern Mediterranean. The number of individuals handling faecal material and disposing of it outside urban centres may have had a considerable impact on the transmission of these parasites and certainly these individuals would be at high risk for infection with intestinal parasites transmitted by the faecal-oral route.

Finally, the reuse of human faecal material as fertilizer is mentioned in Roman texts on agriculture. Varro cites the work of Cassius who stated that human faecal material was second only to pigeon dung as a fertilizer (Varro, *Rust.* 1.38.1–3). Similarly, Columella in his texts on agriculture discusses three types of manure, that from birds, humankind, and cattle; and ranks human excrement after that from birds if it is mixed with other refuse (Columella, *Rust.* 2.14.1–8). Varro even suggests that the latrines of slaves should be placed over the farm's manure pits (Varro, *Rust*. 1.13.4). From cesspits of private latrines, faecal material may have been reused on gardens of the same home or it could have been collected by *stercorarii* and sold as manure (Flohr and Wilson, 2011:147–148). If it was in fact re-used in

this way especially in private gardens where vegetables and fruits were grown, parasite eggs from infected individuals could easily be ingested on unwashed fruits and vegetables thus transmitting roundworm, whipworm, and dysentery in these communities.

5.2.4 Absence of Other Zoonotic Parasites

The absence of certain taxa of intestinal parasites is difficult to interpret because we do not know the degree to which certain eggs may have been lost over time. The samples that have been studied from other published sites are a mix of latrine/cesspit sediment, sewer sediment, occupation layer sediment, pelvic soil, and mummies in Egypt; thus they are broadly similar to what I have studied in *Asia* and *Cyprus* except for the mummified remains from Egypt. It is possible that some parasite taxa are not represented if they were present in lower concentrations that were not picked up during analysis, or if their eggs did not preserve as well as those of roundworm and whipworm. However, it is also possible that zoonotic parasites were just not as common in this region, which is why we have not found them at any of these five sites. This is a similar pattern to what was seen in the wider Mediterranean region from Italy as well. Infection with beef/pork tapeworms, fish tapeworm, lancet liver fluke, *Fasciola* liver fluke, and *Capillaria* generally require interaction with infected animals either by ingestion of poorly cooked liver, meat, or fish, or ingestion of plants that have larvae encysted on them from water contaminated with animal faeces—as in the case of *Fasciola* (Ledger and Mitchell, 2019).

Asia was a heavily urbanized region of the Roman Empire with many large cities including Sardis, Ephesus, and Pergamum (Hanson, 2011). The degree of urbanisation and population density could have contributed to differential transmission of certain parasites. The sites studied in the province of *Asia* are all urban cities where individuals may have had less interaction with animals compared to more rural settings. In conjunction with this, as the number of individuals within a city increased with urbanization, the removal of human waste from the city would have become more of a challenge. This may have increased the transmission and prevalence of faecal-oral parasites if removal approaches could not keep up with the amount of waste generated by inhabitants. While Paphos was not located in the heavily urbanized province of *Asia*, we know it was a prominent urban city in Cyprus with strong Hellenistic roots.

Beef/pork tapeworm and fish tapeworm are acquired when poorly cooked beef/pork or fish are eaten. There are numerous potential explanations for their absence in the eastern Mediterranean; perhaps meat was not widely eaten by all inhabitants of the city (especially those of the lower classes), or meat and fish were generally well cooked. Instead cereals,

legumes, olive oil, and wine have been suggested to be core foods in the Roman diet in the Mediterranean (Garnsey, 1999a; Mitchell, 2015b). Currently there is one stable isotope study done on human remains from Ephesus (Lösch et al. 2014) which has shown that crops such as wheat and barley were likely staple foods, and nitrogen values were low perhaps as a result of legume consumption. Other isotope studies done in Croatia, Italy, and Greece also show a general reliance on C_3 plants with minor additions of fish and terrestrial animals for protein though there is some variation in different sites (see Prowse et al., 2005; Lightfoot et al., 2012; Dotsika and Michael, 2018; McConnan Borstad et al., 2018; O'Connell et al., 2019). Furthermore, cultural influences in the Greek East may have further impacted diet in these cities. A regional exploration of diet in the Roman Empire based on zooarchaeological remains has shown that there were distinct variations in diet in the different provinces. In particular, cities in the Greek East seem to be heavily influenced and consistent with Hellenistic patterns that mainly relied on goat and sheep with the addition of beef and pork, but not at the levels seen in Roman Italy (King, 1999). Zooarchaeological remains from Sagalassos do generally follow this pattern with sheep/goat consistently found in high proportions, though cattle become distinctly more important in the Late Imperial period (Fuller et al., 2012). If consumption of beef and pork was generally minimal, this explains the absence of beef/pork tapeworm. Similarly, while sheep are commonly infected with *Fasciola,* if they were herded away from living areas they may not have posed as much of a risk for infection.

In addition, Hellenistic cooking practices likely influenced how meat was prepared for eating and would impact the risk for infection with foodborne parasites in the region. Eating raw meat (aside from salted products) was compared with the dietary practices of barbarians (Chandezon, 2015:142). However, descriptions of how to prepare salted pork exist, suggesting that it was eaten this way to some extent (Cato, *Agr.* 162.1–3). Preparing meat by salting can kill parasite larvae, however, this requires very high levels of salt for extended periods of time to effectively kill larvae (Khalil, 1969; Adams et al., 1997). Similarly, thorough cooking of meat can effectively kill any parasite larvae, stopping the transmission of parasites like beef and pork tapeworm. The popularity of salted fish or fish sauce (*garum* and *liquamen*) in the Roman Empire is well known, and has been suggested as a potential cause of fish tapeworm infections where this parasite has been found in northern Europe (Mitchell, 2017b). The Mediterranean region of the empire is known for its production of salted fish and fish sauces, and the salting process for preserving fish is thought to have originated in the east and spread to the rest of the Mediterranean (Mylona, 2018). However, it has been suggested that the majority of salted fish products were made from marine fish (Curtis 1991:14; Van

Neer et al., 2010) which cannot act as an intermediate host to *Diphyllobothrium* sp. in this region of the world. Common intermediate hosts for *Diphyllobothrium* sp. in Europe include perch and pike (Scholz et al. 2009). If freshwater fish was ever used to make *garum*, it is unclear if the salting process was adequate to kill fish tapeworm larvae found in freshwater fish. Consumption of raw or undercooked freshwater fish in regions where they were readily available could have caused the cases of fish tapeworm found in the Roman period.

Finally, climate in the Mediterranean likely has an impact on parasite diversity. Though this can be challenging to disentangle from the sociocultural impacts on parasite transmission, we have to consider that it also played a role. The Mediterranean region is generally warmer and drier than northern Europe (Kottek et al., 2006). This may have affected egg survival but at the same time, this warmer climate would increase the rate at which meat and fish would spoil if they were not cooked or preserved with extensive salting. Thus, climate may have also affected food preparation practices that could prevent parasite infections. In conjunction with an overall drier climate at certain times of year, paleoclimate reconstructions have indicated that there was an almost century-long drought in Anatolia from 350–470 C.E. (Izdebski et al., 2016). This drought would have affected the length of growing seasons and the crops that could have been supported. Crop failures may have increased the use and perceived need for fertilizer, some of which could contain human faeces thus spreading faecal-oral pathogens. Paleoparasitological studies from all time periods have shown that the highest taxonomic diversity of parasites is often found in lakeside regions (Ledger et al., 2019a; Maicher et al., 2019). This is likely in part a result of an increase in species that can complete their life cycles using both terrestrial and aquatic animals as intermediate and definitive hosts, potential impacts on egg viability in wetter soils, and increased preservation of parasite eggs in wetter soils.

The ecology and environment of each site is also very important in the parasite species that would have survived and flourished there. Many of the zoonotic species require very specific intermediate hosts and environmental conditions to complete their life cycles. For example although *Fasciola* liver fluke has a worldwide distribution it needs a freshwater environment and appropriate intermediate hosts (many species of lymnaeid snails) to develop. After eggs are shed from the definitive host (humans and animals, especially sheep) miracidia develop in the eggs in the environment for 1–2 weeks then hatch (Garcia, 2016: 499). The miracidia from the eggs penetrate the snail intermediate host where they further develop. Cercariae are shed from the snail into water. Cercariae typically swim in water for ~1 hour or until they attach and encyst on aquatic plants, such as dandelion or watercress (Mas-Coma et

al., 2018). Ingestion of these aquatic plants leads to infection. Since sheep, cattle, and goats are common definitive hosts that can act as reservoirs for *Fasciola*, human infections are more likely in areas where these animals graze near freshwater and human settlements, or their faeces are used as fertilizer for aquatic vegetation (Garcia, 2016: 268). Although zooarchaeological evidence suggests that sheep and goat, both hosts for *Fasciola*, were herded in Anatolia we do not find evidence for the parasite in human contexts in this region. In part, this may be due to climate. The snail hosts for *Fasciola* are found in modern-day Turkey, and the eastern Mediterranean more widely. *Lymnae schirazensis* (one snail host of the parasite) has been suggested to have originated in the Near East and spread from there (Bargues et al., 2011). However, the snails need freshwater environments to survive. Snail populations may have been maintained around rivers, man-made irrigation channels, and standing water in rainy seasons. The drier summers in Anatolia and droughts in fourth and fifth centuries may have decreased snail populations at certain time periods while wetter climates with fewer temperature fluctuations can maintain transmission for more of the year. In western Europe, there is often biseasonal transmission, with some transmission of the parasite in spring and high transmission in autumn (Mas-Coma et al., 2018). In Scotland infected snails have been recorded from April through October (Ross, 1977). Modern work comparing sheep *Fasciola* infections in Europe found that 62% of farms in Ireland were positive, compared to 8% in southern Italy and 4% in Switzerland (Rinaldi et al., 2015). Modern mappings of risk for *Fasciola* transmission across Europe and the Near East based on climate generally follow the distribution of *Fasciola* eggs found in Roman period sites (see Fig. 3 in Caminade et al., 2015). This may explain why 6 of the 8 cases of Fasciola liver fluke from the Roman period are found north of the Alps (Figure 15).

It is important to consider that differential preservation of parasite eggs from different taxa may be contributing to the patterns observed. The eastern Mediterranean region of the empire has a much warmer and drier climate than that in northern Europe which has an impact on parasite species that can survive there but likely also has an impact on the preservation of parasite eggs. There is little experimental evidence for how specific soil conditions impact parasite egg survival. However, we may expect that parasite eggs survive better in waterlogged conditions; similar to other archaeological materials. Indeed a high diversity of parasite eggs have been recovered from waterlogged sites in northern Europe and these eggs are very well preserved based on the morphology of the eggs (Maicher et al., 2017; Ledger et al., 2019a; Maicher et al., 2019). This may be part of the reason why a higher species diversity has been recovered from Roman sites in northern Europe. However, we must also consider that the eggs from different species will preserve differently. For example,

roundworm and whipworm are known to be quite robust and have been found across Eurasia from the Neolithic period onwards in a variety of climates (Anastasiou, 2015). The structure of the egg shells is likely to be very important in this differential preservation. The egg shells of helminth eggs have multiple layers, one of which is a chitinous layer (Wharton, 1980). Different species have varying thicknesses of this chitinous layer, with varying orientations of protein fibrils (Wharton, 1980). As chitin is known to be resistant to degradation and its purpose it to provide strength this likely impacts egg preservation (see section 7.3 for a further discussion). Therefore, I would expect that eggs, such as *Taenia* sp. and *Dicrocoelium dendriticum*, with thick chitinous walls similar to roundworm and whipworm could preserve in the same sites these eggs have been found in, if they were present.

5.2.5 Summary

Overall in the eastern Mediterranean we are seeing a similar pattern of parasite taxonomic diversity as is seen in Italy, a predominance of parasites transmitted by the direct faecal-oral route and an absence of zoonotic species. So far in the Mediterranean region almost exclusively soil-transmitted helminths and protozoa causing severe diarrhea or dysentery have been found. This is in contrast to the much wider taxonomic diversity found in northern Europe such as that in the frontier regions of the empire which will be discussed in the following sections.

There are numerous social, environmental, and ecological explanations for the transmission of these parasites. An examination of archaeological and historical evidence that describe the design of latrines and sewers, some aspects of their use, common methods for disposal of human excrement, and reuse of human excrement show that there are numerous pathways for people in Roman communities in the Mediterranean to come into contact with faecal material or to have it contaminate their food. Furthermore, historical and archaeological evidence for dietary preferences and cooking practices provide some insights into the absence of foodborne parasites such as beef and pork tapeworm, which have been found in other regions. Climate is also a strong determinant of parasite transmission, and particularly may have affected snail populations and transmission of *Fasciola* liver fluke.

5.3 The Frontier Regions

The focus of this section is on the frontier regions of the Roman Empire along the Rhine and Danube rivers. The subsequent section will focus on Roman Britain specifically, which is also considered a frontier region but will be discussed separately. The Rhine and Danube rivers served as the borders of the Empire in central Europe and the frontier provinces in this region

include *Germania Inferior* and *Superior*, *Raetia*, *Noricum*, *Pannonia Inferior* and *Superior*, *Moesia Inferior* and *Superior*, and *Dacia*. The Roman frontiers have often been studied from a military perspective because as bordering regions they had a large military presence to protect from rebellions and invasions (Elton, 1996). Forts that were built along the edges of the Roman Empire attracted civilians and in many places settlements were also established in these areas. These areas also served as links for trade between the empire and regions beyond it.

5.3.1 Previous Work

Evidence for disease amongst the inhabitants of the frontier provinces in Central Europe is not as numerous as that in Roman Britain or the Mediterranean region. Thus the evidence for intestinal parasite infection in these provinces is an important addition to the picture of what daily life was like in these areas of the empire. Previous parasitological work done on frontier provinces along the Danube and Rhine rivers has contributed evidence for intestinal parasites in modern-day Austria, Belgium, Germany, Netherlands, and Switzerland. However, we will discuss this evidence in relation to the Roman province that these settlements were found in. From *Germania Inferior* the sites of Alphen on the Rhine, Valkenburg Army Camp, and Utigeest have been studied. *Trichuris* sp. eggs were found at all three sites. At Alphen on the Rhine eggs of *Ascaris* sp. and *Taenia/Echinococcus* sp. were also found in the 1st c. CE latrine studied (Kuijper and Turner, 1992). At Valkenburg occupation layer samples also contained eggs of *Ascaris* sp. (Jansen and Over, 1966).

In *Germania Superior* six sites have been studied. A highly diverse set of parasites was found in samples from Horbourg-Wihr. First to third century CE samples from pits, latrines, and ditches at the site provided evidence for *Ascaris* sp., *Capillaria* sp., *Dicrocoelium* sp., *Diphyllobothrium* sp., *Fasciola* sp., *Macracanthorhynchus* sp., *Oxyuris equi*, *Taenia/Echinococcus* sp., and *Trichuris* sp. (Dufour, 2015). However, without further details of the samples studied it is impossible to know what proportion of these parasites were from human or animal infections. Especially with the findings of parasites that infect animals such as *Oxyuris equi* and *Macracanthorhynchus* sp., a number of these taxa must be from animals at the site. In an unknown sample type from the site of Andilly-en-Bassigny eggs of *Ascaris* sp., *Fasciola* sp., and *Taenia/Echinococcus* sp. were found (Dufour, 2015). At the other four sites, all samples were from 1–2nd c. CE latrines and contained eggs of *Ascaris* sp. and *Trichuris* sp. (Hänggi et al., 1989; Hufschmid and Sütterlin, 1992; Goppelsröder and Sommer, 1996; Jauch, 1997; Le Bailly et al., 2003c).

The province of *Belgica* has also been well studied previously with evidence from five sites, Arlon, Mageroy, Reims, Metz, and Belginum. Previously studied samples from the city of Arlon include soil samples from vats and pits of a fuller's shop which contained eggs of *Ascaris* sp. and *Trichuris* sp. (Defgnée et al., 2008). At Mageroy soil samples from a latrine tested positive for *Entamoeba histolytica* using ELISA (Le Bailly and Bouchet, 2015), the only evidence for protozoan infection in this region. From Reims, Metz, and Belginum eggs of *Ascaris* sp., *Capillaria* sp., *Dicrocoelium* sp., *Diphyllobothrium* sp., *Fasciola* sp., *Taenia* sp., *Toxocara* sp., and *Trichuris* sp. were all identified in a variety of samples including cesspits, pits, wells, and occupation sediments (Dittmar et al., 2002; Le Bailly et al., 2003b; Le Bailly and Bouchet, 2010; Dufour, 2015). While the diversity of taxa at these sites is quite high compared to other provinces along the Rhine and Danube it should be noted that the diversity of sample types studied is also higher than in other provinces in the region.

Other evidence for the frontiers comes from one site in each of the provinces of *Raetia* and *Pannonia Superior*. At Künzing in *Raetia* eggs of *Trichuris trichiura* were identified in a pit whose use dated between 140–250 CE (Specht, 1963). In latrine and sewer samples from the 1st–2nd c. CE eggs of *Ascaris lumbricoides, Taenia* sp., and *Trichuris trichiura* were reported at the site of Carnuntum in *Pannonia Superior* (Aspöck et al., 2011; Petznek, 2018).

All of these sites are located in provinces in frontier regions however, not all of them are strictly frontier sites. A frontier site in the Roman Empire is one that is located directly on the borders (frontiers or *limes*) of the empire and thus had a strong military presence (Whittaker, 2004; 5–6; Breeze, 2018). Of the sites studied in the frontier provinces those that are actually frontier sites include: Alphen on the Rhine, Valkenburg, Carnuntum, and Künzing. The newly studied sites Lauriacum and Viminicium are also frontier sites.

5.3.2 New Results

Work from this dissertation contributes additional evidence for parasitic infection in the frontiers of central Europe from human contexts at the site of Arlon in the province of *Belgica*, as well as the first evidence for parasites from the provinces of *Noricum* and *Moesia Superior*. Soil from a latrine at the site of Arlon in *Belgica* contained the eggs of *Ascaris* sp., *Capillaria* sp., *Dicrocoelium dendriticum*, and *Trichuris trichiura.* Pelvic soil from skeletons buried between the 3rd–4th c. CE at Lauriacum, in the province of *Noricum*, did not contain any preserved parasite eggs. At the site of Viminacium in the province of *Moesia Superior*, a coprolite collected from the bath complex contained eggs of *Ascaris* sp. and *Trichuris trichiura*. These findings are generally in line with previously published work from the frontier provinces. In *Belgica* there is a more diverse range of intestinal parasites, similar to

results from sites in central and northern Europe, while in *Moesia Superior* the presence of *Ascaris* sp. and *Trichuris* sp. is similar to what has been found in the wider Eastern Mediterranean region.

The highest diversity of parasites in this region is in *Germania Inferior*, *Germania Superior*, and *Belgica*, while the exclusive presence of soil-transmitted helminths in *Moesia Superior*, *Pannonia Superior*, *Noricum*, and *Raetia* is more in line with what is found in the Mediterranean region. It first needs to be acknowledged that this pattern may be a result of the higher number of sites studied in *Germania Superior*, *Germania Inferior*, and *Belgica*. Only a single site has been studied in each of the other provinces and indeed there are sites in *Germania Inferior*, *Germania Superior*, and *Belgica* that only have *Ascaris* sp. and *Trichuris* sp. Furthermore, many of the sites in *Belgica* are not predominately military sites, rather they include a number villas and villages (*vici*). At the sites that are frontier sites, directly on the borders, only *Ascaris* sp., *Trichuris* sp., and *Taenia* sp. have been found.

5.3.3 Soil-Transmitted Helminths

The presence of soil-transmitted helminths and other sanitation related parasites such as *Entamoeba histolytica* can be attributed to similar cultural and social practices that have been discussed in relation to Italy and the eastern Mediterranean. As discussed in the previous two sections multi-seat latrines found in the Roman Empire were common in the East and Italy and were likely adopted from their Greek counterparts. However, beginning in the 1st c. CE latrines are found much more widely in Gaul and the frontier provinces of the northeast, and in the 2nd c. CE public latrines become more common in the region (Bouet, 2009:157). Published examples of multi-seat latrines in bath complexes in Germany come from Colonia Ulpia Traiana (Zieling, 2018), Aachen (Schaub, 2018), and the *vicus* of Bonn (White, 2018). Dodt (2018) describes a number of public latrines associated with bathhouses from Heerlen, Zülpich, Soller, and a further seven from private bathhouses at *villae*. Most of these have flushing mechanisms where water from the *frigidarium* was channeled underneath the latrine seats to wash away faecal material. A rather opulent latrine, for German standards, has been reported from Rottenburg. It had seating for 35–54 people, plastered sandstone columns, painted walls, and a stone basin for washing (Hoss, 2018). Additionally, a latrine was found in the garden area of a house at the *vicus* of Bonn (Andrikopoulou et al., 2018). There are few examples of private latrines published from the frontier provinces, however an interesting example comes from Nijmegen where cesspits that likely had wood structures built over them were found in the gardens of most land parcels (Heirbaut, 2018), a similar placement of cesspits in gardens was found at the *vicus* of Oberwintherthur in Switzerland (Jauch, 2018).

Bouet (2009) also provides a catalogue of latrines from *Germania Superior*, *Germania Inferior*, and *Belgica* many of which are private latrines or pits behind houses but there are also larger communal latrines associated with bath complexes and larger villas. The floors of some of these latrines are also described and can be earth-beaten, cement, or wood; often dependent on what material the building itself was made out of, and whether the latrine was a stand-alone building or part of a larger complex (Bouet, 2009:137). Earth-beaten floors are more common in simple private latrines. Unfinished floors have been linked to an increased risk for soil-transmitted helminths in modern communities (Worrell et al., 2016) and may have contributed to the transmission of soil-transmitted helminths in these provinces. Especially in a latrine where floors could easily be contaminated with faecal material from various users, this may have increased the risk for transmission if floors were not cleaned regularly.

Chamber pots would have also been widely used in these provinces, as they were in the Mediterranean. Numerous examples and shapes are described by Petznek (2018) and Bouet (2009:68). Interestingly it is noted that there is regional pattern to the different shapes that likely relate to differences in dress, with men of Celtic tribes wearing pants that would require sitting while defecating as opposed to tunics which could allow for squatting. Nevertheless, the potential for spread of soil-transmitted helminths as a result of dumping chamber pots into the street or other collection areas is similar to that in the Mediterranean region.

5.3.4 Diet and Animal Interactions

What is notable at these sites compared to the Mediterranean is the presence of parasites that are acquired from undercooked meat or interactions with animals more generally. While *Ascaris* sp. and *Trichuris* sp. have some potential to be zoonotically acquired they are not necessarily zoonotic like some other species such as *Taenia* tapeworms and fish tapeworm (Ledger and Mitchell, 2019).

The provinces along the Danube and Rhine rivers were sparsely populated before the stationing of military legions and the growth of towns, this would have necessitated an increase in food production and import of foodstuffs (Whittaker, 1994: 99). There is evidence from various sites in the northeastern provinces of the empire suggesting that local animal husbandry practices exhibited measurable continuity after stationing of Roman military troops and urbanization (Groot and Deschler-Erb, 2017; Trixl et al., 2017). Most of the people living in these provincial regions of the empire would have been local populations to begin with. The Roman individuals would have consisted of the military troops, the governor and

administrative officials, and merchants and families who followed military troops (Elton, 1996:15). Continuation of pre-existing foodways may have been necessary to support a growing population until imports from elsewhere in the empire were well established. Many foodstuffs and items may have been imported to meet the preferences of the Roman military and administrative individuals, until they became a regular items produced in these communities.

In *Belgica* zooarchaeological assemblages have suggested a diet dominated by beef as opposed to the heavier reliance on pork seen in Italy and the Mediterranean (King, 2001). Similarly in *Germania Inferior* and areas along the Rhine, King (2001) has reported cattle bones making up 60% of the assemblages. When these data are compared to pre-Roman settlements it appears that cattle was also the main domesticate before the Roman conquest. Furthermore, changes to husbandry practices in different settlements in the provinces were varied, with some maintaining foodways very similar to the pre-Roman period (Valenzuela-Lamas and Albarella, 2017). The reliance on cattle may explain the eggs of *Taenia* sp. found at Carnuntum, Belginum, Alphen on the Rhine, and Andilly-en-Bassigny. If any of this beef was eaten raw or undercooked, infection with beef tapeworm (*Taenia saginata*) could occur. Certainly, if we relied upon the opinions of elite writers from the Mediterranean we would be informed that those living in the farther northern areas of the empire such as the Gauls ate very differently to elite Romans. These individuals living in northern regions of the empire are said to have relied on milk and the flesh of many animals available to them sometimes even to be eaten raw (Garnsey, 1999b). Despite the reliance on cattle shown by zooarchaeological remains, *Taenia* sp. is only found at these four sites in the frontier regions indicating a low prevalence of the tapeworm, that meat was generally well cooked, or that meat was not eaten as frequently as the zooarchaeological assemblages on their own may suggest.

Even within Roman settlements in the northeastern region of the empire the zooarchaeological remains vary, likely as a result of local ecology (Trixl et al., 2017). For example, Deschler-Erb (2017) analysed zooarchaeological remains from three fortresses in Roman Switzerland finding that proportions of wild animal remains ranged from 2–17% with the higher end likely reflecting hunting among military personnel and populations of deer, elk, beaver, and wild boar in the area. Similar variations are seen in different types of settlements, with some *villae* having higher proportions of wild animal remains likely due to local populations of deer and popular hunted animals. Military sites in Switzerland and the Netherlands have been found to have higher proportions of pig remains than cattle, in

opposition to other rural and urban sites in the area, suggesting that the Roman military diet may be closer to Roman diets in the Mediterranean (Groot, 2017; Groot and Deschler-Erb, 2017). We may then expect that dietary patterns at sites along the frontiers may be reflective of the diet of the previous Celtic Iron Age tribes in the region with additions taken from the typical Roman diet in the Mediterranean. For Viminacium that would be the Scordisci in Serbia, the Treveri around Arlon in Belgium, and the Hallstatt culture in pre-Roman Austria where Lauriacum was located. Further research on these Iron Age communities would be very valuable.

Viminacium, one of the sites studied as part of this research, is located farther south than those in Austria and Belgium and thus it is worth considering it separately. This is the first palaeoparasitological evidence from a site in Serbia in any time period so unfortunately there is very little comparative data. Viminacium was established as a legionary camp that had two civilian settlements around the camp. Viminacium was granted municipal status under the reign of Hadrian. As a site located much closer to the Mediterranean region with a consistently heavy military presence it may be expected that it was influenced more heavily by cultural practices in the Mediterranean. Certainly, the presence of solely *Ascaris* sp. and *Trichuris trichiura* is similar to what was found in *Asia* and *Cyprus* (see previous section). The settlement was located on fertile land and a number of crops were grown around the legionary camp and civilian settlements. Thus agriculture was likely an important part of the economy of the city and these crops would have been a necessary food source for the legion stationed there. There is evidence for various crops including wheat, millet, barley, rye, lentils, broad-beans, common vetch, and reliance on local fruits (Medović, 2013). The presence of the bath complex and communal latrine where the coprolite studied came from attests to the influence of Roman culture from the Mediterranean.

Cereals, wine, and olives are often referred to as staples in the Roman diet (Garnsey, 1999a:13; Purcell, 2003) however the types of cereals and availability of wine and olive oil would have varied immensely based on ecology and distance from production areas (Erdkamp, 2015). For the typical individual living in the Roman Empire meat and fish were actually not a consistent or major part of the diet but fruits, vegetables, cereals, and pulses would have been the major contributors to caloric intake (Garnsey, 1999a:18; Craig et al., 2009). Though fish was part of the diet of many individuals in the Roman empire, *Diphyllobothrium* sp. eggs have only been found at six Roman period sites, none of which are in the provinces along the Rhine and Danube river and no additional evidence for fish tapeworm was found at the sites studied as part of this dissertation. If freshwater were

consumed widely in this region perhaps they were often well cooked. That being said, fish sauce was a popular condiment in the Roman Empire and it was widely traded within the empire and local variants were produced in Gaul, Britain, and Belgium (Van Neer and Lentacker, 1994; Ven Neer et al., 2005). It is possible that the fish sauce imported and produced in the region was made primarily using marine fish which are not an intermediate host for the parasite in this region of the world.

5.3.5 Summary

In summary, the pattern of parasites found in the frontier provinces of the northeastern Roman Empire is more diverse than that in the Mediterranean region. Though the only existing Iron Age data come from Austria, the species found at that site are similar to those found at Roman period sites. Additional samples may show that there was a continuity of Celtic patterns of parasite infection very similar to what has been observed for cultural practices such as diet. Furthermore, the ecology and environment of the settlements in these regions would have had strong influences on the species of parasites that could survive and flourish there and it does not appear that changing cultural practices as a result of Roman occupation drastically changed the types of intestinal parasites that people were infected with. There is very little data that is well dated, thus to get a better sense of how Roman occupation influenced parasite infections it will be necessary to study further samples from various time periods and from additional Iron Age and Medieval contexts.

5.4 *Britannia*

The majority of previously published palaeoparasitological work in the Roman period is on sites from Roman Britain (see section 2.3). However, most of this work has used samples from pits, occupation layers, and some latrines. The samples analysed for this dissertation came from the pelvis of skeletons in an attempt to gather more information about intestinal parasite infection at an individual level. Previous work has revealed evidence for six different taxa of intestinal parasites in Roman Britain, namely *Ascaris* sp., *Dicrocoelium* sp., *Diphyllobothrium* sp., *Fasciola* sp., *Taenia* sp., and *Trichuris trichiura*.

5.4.1 New Results

The two sites studied as part of this dissertation were the Roman cemetery at West Smithfield, London and Winterborne, Dorset. From West Smithfield, pelvic soil samples from 29 individuals with ages ranging from 6–45 years were analysed. Three adults, both male and female, were found to be infected with *Ascaris* sp. None of the five individuals studied from

Winterborne, Dorset had parasite eggs in their pelvic soil. As such the data do not allow us to determine if there were any patterns in infections at the individual level. For example, if certain types of parasites were more common in males compared to females, or in children compared to adults. This type of study would be useful for confirming if the distribution of parasite infections in past populations was similar to what is observed in modern populations. In modern epidemiological studies the highest intensities of helminth infections are often seen in school-aged children (5–15 years), while rates of infection are often similar between genders (Hotez et al., 2006). Analysis of these samples does give us additional evidence for roundworm infection in Roman London.

5.4.2 Patterns in Roman Britain

As the region with the largest amount of evidence, the parasite data from Roman Britain was analysed more closely for patterns of infection by site type and sample type (Figure 58; for raw data see Appendix B.6). The site type was broken up into three categories: military, urban, and rural. A military site was any site that was a camp, fort, or fortress (Ambleside, Bearsden, Carlisle, and Poundbury); an urban site was any site designated as a large town or *colonia* where samples did not come from areas that would have been primarily occupied by the military (Leicester, Lincoln, London, and York); and a rural site was other small settlements not associated with a main military camp, fort, or fortress (Owslebury). Of course it should be acknowledged that many military sites in the Roman Empire had small civilian settlements located nearby thus, these categories are a general assignment based on the primary activities that occurred at the site. Sample types were based on the description of the samples by the original authors; it is possible that some pits are actually cesspits but were not identified as such during excavation. The burial sample type includes pelvic soil and soil from inside coffins.

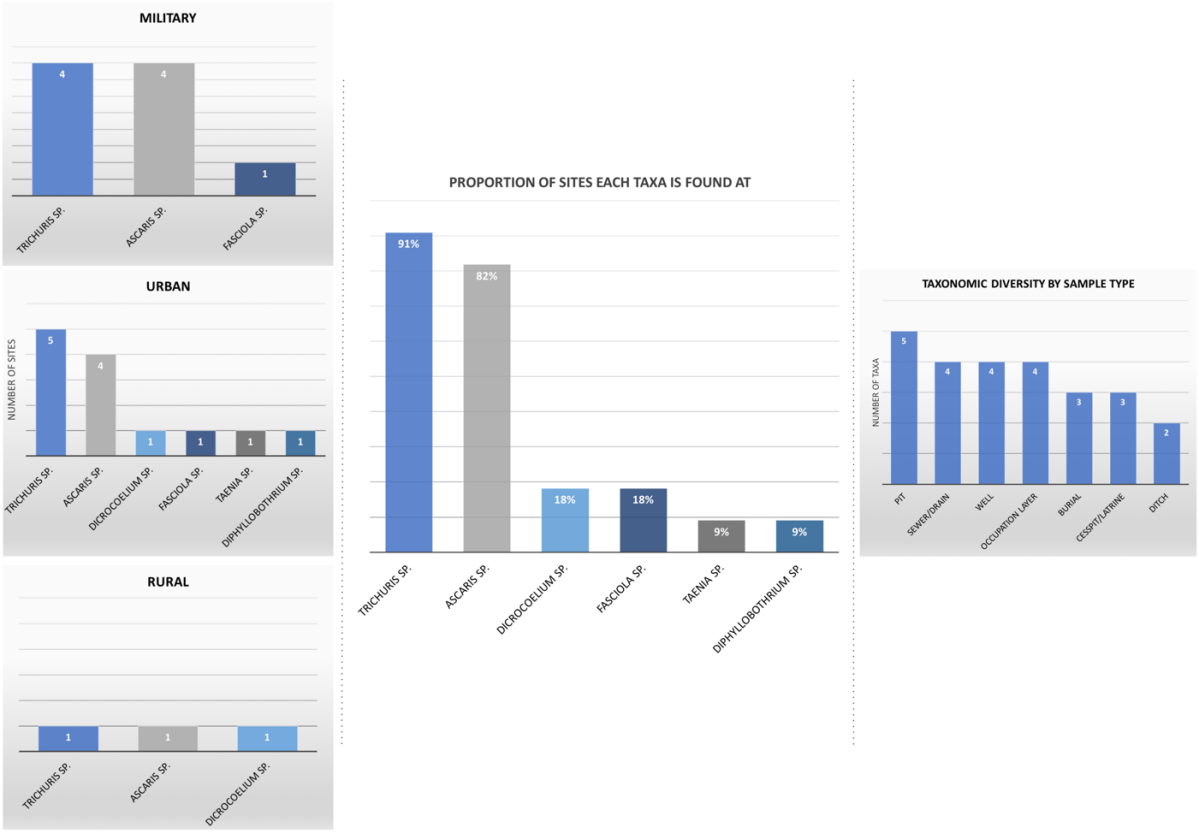

Figure 58: Distribution of parasite taxa at Romano-British sites. Overall proportion of sites in which each taxa of parasite was found (centre); number of sites each taxa is found in by category of site (left); number of taxa found in different sample types (right).

As can be seen in Figure 58 the most common parasites identified in Romano-British sites are *Ascaris* sp. and *Trichuris* sp.; with *Ascaris* sp. eggs identified at 82% of sites and *Trichuris* sp. at 91%. *Dicrocoelium* sp. and *Fasciola* sp. were the next most commonly identified parasites, but were found at a much lower proportion of sites; 18% for each. The least common taxa were tapeworms; *Diphyllobothrium* sp. and *Taenia* sp. eggs were both found at 9% of sites. When we look at the different site types, *Ascaris* and *Trichuris* are the most commonly identified eggs in military and urban sites, and they have been identified at the one rural site studied. This is in line with what has been found in the rest of the Roman Empire (see section 2.3.6). Urban sites have the highest taxonomic diversity of any site type. *Fasciola* sp. eggs were identified at military and urban sites but not in rural sites. *Dicrocoelium* sp. eggs were identified in urban and rural sites but not at military sites. No statistical analysis of these comparisons has been done because the sample sizes are very small.

5.4.3 Soil-Transmitted Helminths

The high proportion of sites where roundworm and whipworm were found is not surprising after the patterns already discussed from *Italia*, *Asia*, and the other frontier provinces. Soil-transmitted helminths were widespread in the Roman Empire despite regional variations in cultural practices, variations in sanitation infrastructure, and urbanisation. The evidence for latrines in *Britannia* varies based on the type of site. In military camps and forts, latrines were often set up even on a semi-permanent basis. In temporary camps, latrines would have been trenches dug into the ground that could then be filled over (Hobson, 2009:33). The pit that was studied from Ambleside may have been one of these temporary latrines. The specific context of this pit is not described in the report on parasites found in the pit (Jones, 1985). In more permanent forts and fortresses, latrines (private and multi-seater) were built either as part of bath complexes or independently (Revell, 2007; Hobson, 2009:35; Rushworth, 2009:222). Less work has been done looking at the location of private latrines in typical Roman houses in Britain. Most information on private latrines comes from elite villas, large towns, and military settlements. We may expect that many modest houses did not have private latrines, or had cesspits located outside the house similar to what has been found in *Germania Inferior* (see 5.3). It is also likely that chamber pots were widely used, and in a region reliant on agriculture, excrement may have been collected in dung heaps and reused as fertilizer for crops or small gardens.

Housing layout was also different from what is seen in the urbanised cities studied from *Asia* and *Italia*. Many rural farmsteads and buildings were constructed primarily of timber (Perring, 2002:83). Other buildings such as bathhouses, and later forts, were constructed of stone or brick (Wacher, 2000:20, 56). It is not until the mid 2nd c. CE when masonry becomes more widespread in Roman Britain (Perring, 2002:106). This is in contrast to the marble, stone, terracotta, mortar, and brick found in the Mediterranean quite widely. Many early Roman buildings in Britain had earth-beaten floors, these have even been called standard (Perring, 2002:126). Where stone was readily available, stone-flagged floors have been found in working areas of houses and lime-based cement floors were also common in nicer houses. Mortar and mosaic floors are found mostly in higher class buildings such as the villa at Fishbourne and some houses in London (Perring, 2002:32). Flooring and building techniques can influence pathogen transmission, and in particular soil-transmitted helminths. Worrell and colleagues (2016) found that having finished household floors was a protective factor against soil-transmitted helminths in modern populations in Kenya. Earth-beaten floors may have been harder to wash compared to those finished with materials such as stone or

concrete, and any contamination of the living space with faecal material on shoes, from chamber pots, or from numerous flies known to be present in Roman settlements could have resulted in helminth eggs surviving in the home.

The economy of Roman Britain was heavily based on agriculture as it was in the preceding Iron Age (Millett, 2005:38). Agricultural improvements were happening in the area before the conquest by the Romans, and continued with the introduction of new agricultural equipment by the Romans. Production likely expanded after Roman occupation in response to increased population sizes brought on by the presence of large military troops, an estimated 55,000 people at its peak (Millett, 2005:37), and export to other areas of the empire. This meant that previously unused land may have started to be used as agricultural land in this period (Millett, 2005:38). Wacher (2000:20) has also estimated that 1000 km^2 of woodland was deforested over the first 150 years of occupation both to supply wood for building new forts and settlements, and for fuel. This area would have also provided additional land for agriculture, neighboring newly established forts or settlements. With most towns relying on agriculture and farmland, it is expected that even people living in more urbanised centres may have had continual interaction with farm animals. Nonetheless most of the population is estimated to have lived in rural settlements with an agrarian focus similar to the preceding Iron Age. Millett (2005:36) estimated a rural population of 3.6 million and only 240,000 in larger towns. This may have been a factor in the presence of zoonotic parasites found in Britain but not in large cities in *Asia* or the wider Mediterranean.

5.4.4 Liver flukes

After roundworm and whipworm, the next most common parasites found in Roman Britain are the liver flukes, *Dicrocoelium* sp. and *Fasciola* sp. While both of these genera include species that can infect humans they also often cause pseudoparasitism (ingestion of eggs that does not result in infection). Certainly liver is included in many Roman recipes from the *Apicius* (7.3.1-2 and 7.10.1-2) and in particular it is listed under ‘luxury dishes’. The liver of chicken, sheep, and pigs are specified in recipes, but there are also recipes where the type of liver is not specified. In most cases the recipes call for cooking by boiling or frying (*Apicius* 8.8.5). It seems unlikely that these preparations of liver were common in the Roman Empire and may have been consumed more often by elite individuals, though liver may have been regularly consumed by anyone. *Dicrocoelium* sp. eggs have only been found at sites in Britain, France, and Israel; and *Fasciola* sp. has been found at sites in Britain, France, and Italy. It is also possible that dietary practices in these regions were more likely to contain raw or undercooked liver, that these are cases of pseudoparasitism if animal faeces contaminated

living spaces, or animal faeces were dumped in latrines, cesspits, and other refuse pits. The evidence for *Fasciola* and *Dicrocoelium* in Britain was found in occupation layer soil, pits, and ditches; none of which can be confidently linked to human ingestion of eggs or human infection. However, the presence of eggs in living areas at all site types indicates that humans were at risk for infection with these parasites. Liver was eaten in the Roman period and known to be a nutritious food. Thus, for individuals whose main source of income came from agriculture and pastoralism, selling or eating the liver of animals may have been common even if not prepared for the elite tastes that the *Apicius* catered to.

Fasciola hepatica is the most common species of *Fasciola* to infect humans and it is acquired through the consumption of aquatic plants such as watercress, dandelion, and spearmint which have larvae encysted as metacercariae on them as a result of human or animal faeces finding their way into water (Mas-Coma et al., 1999; Garcia, 2016). Common animals that carry *Fasciola hepatica* are sheep which were known to be herded in Roman Britain and were the most common herded animal in Iron Age Britain. The presence of *Fasciola* sp. eggs in occupation layer soils at Carlisle and a cesspit at Leicester may reflect local consumption of aquatic plants and proximity to sheep.

5.4.5 Tapeworms

The only evidence for tapeworms, both fish and beef/pork, comes from urban centres, in particular London. This may be a result of access to a larger variety of foods and exotic foods in major centres. Archaeobotanical evidence from 514 sites in Roman Britain has shown that imported foods that were new to Britain in the Roman period were more often found in major towns such as London (van der Veen, 2008). These include things like figs, grapes, mulberry, pine nut, olives, and lentils. It appears that people living in major cities like London had greater access to more exotic foods that were imported from elsewhere in the empire. This may have included other items such as fish sauce and preserved meats which could have been responsible for tapeworm infections. The only evidence for pre-Roman parasites in Britain comes from the Bronze Age site of Brean Down (Jones, 1990) and the Must Farm settlement studied here (Ledger et al., 2019a). *Taenia* sp. was not found at either of these sites though *Diphyllobothrium* sp. was found in coprolites and soil samples from Must Farm indicating its previous presence in the Fens of England.

The most common species of fish recovered from Roman sites in Britain are eel, herring, plaice, cyprinid, and salmonid (Locker, 2007). Cyprinids are exclusively freshwater fish while eel and salmon spend part of their life in freshwater. *Diphyllobothrium* sp. can be found in salmon and cyprinids thus consumption of these fish could result in infection if they

were eaten raw or undercooked. Marine fish was generally preferred over freshwater fish in the Roman period though it was more expensive and there is ample evidence for freshwater fish consumption especially amongst the lower classes (Locker, 2007). There is less evidence for fish consumption in Iron Age Britain, both based on zooarchaeological remains and stable isotope data (Jay and Richards, 2006; Dobney and Ervynck, 2007; Jay, 2008). Fish consumption then increases in the Roman period. Fish remains are found throughout sites in Roman Britain though fish, and fish tapeworm, becomes much more common in Britain in the Medieval period as a result of the spread of Christianity (Cool, 2006:105; Locker, 2007; Mitchell, 2015a).

If fish sauce was produced from freshwater fish in this area of Britain it is possible that it could transmit fish tapeworm if salting conditions were not adequate to kill larvae. Recovery of fish bones and amphora, common shipping containers, has given evidence for importation of fish sauce and pickled fish from Iberia starting in the Iron Age and increasing into the Roman period (Cool, 2006:59). These amphorae become less common in the 2nd c. CE, it has been suggested that this may be a reflection of fish sauce production in Britain (Cool, 2006:61). The only evidence for fish tapeworm in Roman Britain comes from London, there has been evidence for *garum* or *liquamen* production at a waterfront site in London (Bateman and Locker, 1982). However, the fish remains found suggest that garum was made from herring and splat which are not common intermediate hosts of *Diphyllobothrium* sp. Cool (2006:62) has suggested that fish sauce was mainly consumed by the military and in urban sites, and it was less popular at rural sites and certainly not consumed regularly by most people living in Roman Britain.

The presence of *Taenia* tapeworm, also in London, may represent infection with beef tapeworm (*Taenia saginata*) or pork tapeworm (*Taenia solium*). Though the dominant faunal remains in Iron Age sites in Britain are sheep, in the Roman period there is a shift to high levels of cattle across the province and high levels of pig at some sites, mainly military and urban (King, 2001). As one of the major urban centres in Britain, London is one of the sites where higher proportions of pig remains would be expected and some faunal assemblages show this (Maltby, 2016). Sheep or goat were more common on rural sites, showing continuity with Iron Age sites (King, 1978). Numerous cattle scapulae have been found with a hole through the centre which suggests that shoulders were hung to be dried or smoked (Cool, 2006:89). Smoking and drying are not always effective at killing parasite larvae and this could be another practice leading to beef tapeworm infection. High levels of pig remains but also the practices of preserving cattle mean it is possible that the *Taenia* sp. eggs found in

London could have come from infective beef or pork. Pigs were also a major source of meat in the Mediterranean region however *Taenia* sp. eggs have not been found in the Mediterranean region in the Roman period so far. Cooking practices, levels of meat consumption as well as the fact that more samples have been studied in Britain compared to much of the Mediterranean all likely contribute to his pattern to some extent.

One point to consider when comparing the parasite remains from Britain to other regions of the Empire is the exceptional organic preservation at some of these sites where waterlogged conditions have resulted in excellent preservation of archaeobotanical remains, wood, and bone (Wilcox, 1977; Millet, 2005:20). Other sites such as York, London, and Carlisle have also provided waterlogged deposits. It is unclear where the distinction is between increased organic preservation due to these wet conditions and increased support of the life cycles of a more diverse set of parasites both in antiquity and modern-day. There is little comparative evidence for intestinal parasites in earlier time periods in Britain but work discussed as part of this doctoral research in the Fens during the Bronze Age shows that the freshwater aquatic environment of the Fens is an ideal ecological niche for a range of parasite taxa (Ledger et al., 2019a). Despite this I would expect that *Taenia* eggs, with their thick chitinous walls, would be equally robust as *Ascaris* and *Trichuris*. All of these species do not rely on hatching in the environment for their life cycles, thus should have similar rates of preservation.

5.4.6 Summary

In summary, the parasite evidence from Romano-British sites follows the pattern seen in other regions where the soil-transmitted helminths, whipworm and roundworm, are the most frequently identified. However, in Britain we also see cases of zoonotic parasites including liver flukes and tapeworms. At least one species of liver fluke, either *Dicrocoelium* sp. or *Fasciola* sp., was found at each site type and both species were found together at urban sites. The presence of liver flukes may be a result of consumption of liver from various animals or contamination of sites with animal faeces. Tapeworms including, beef/pork tapeworm (*Taenia* sp.) and fish tapeworm (*Diphyllobothrium* sp.) were only found at urban sites which may reflect a more diverse diet or access to more exotic foods at these sites.

6 Wider Trends in Parasitic Infection

From the different regional foci a few trends start to become evident. Soil-transmitted helminths are the most common parasites identified in each region and they are found in all regions of the empire. Their transmission in these communities is likely reliant in part on sanitation infrastructure design and use. Furthermore, approaches to waste disposal and reuse, as fertilizer for example, can be a major contributor to transmission.

Another trend that starts to appear is the presence of foodborne or zoonotic parasites primarily in regions north of the Mediterranean. Taxa such as liver flukes (*Dicrocoelium* sp. and *Fasciola* sp.), tapeworms (*Taenia* sp. and *Diphyllobothrium* sp.) and others such as *Capillaria* sp. are found in the frontier regions of the empire and in France but have not been identified from most of the Mediterranean sites studied. This may be a result of diverse dietary practices, locally defined culinary preferences, variations in animal husbandry practices, and ecology.

Alongside social factors, cultural practices, and environmental factors—including the design of cities and infrastructure, climate and ecology likely has an important role to play in parasite presence in these regions. As many parasites spend a part of their life cycle outside of a host, their life cycles as well as preservation are reliant on local ecology. Many more sites in the northern provinces have waterlogged contexts, compared to the Mediterranean region, which could result in better egg preservation (for a further discussion of preservation see section 7.3). It is important to note that this may in part account for the higher diversity of parasite species that has been found in these provinces in past and current research, however the climatic impact on egg preservation is largely unknown. Climate and local ecosystems are important for parasite survival and reproduction, and latitude in particular has been shown to be correlated with parasite diversity. As latitude increases so does helminth species diversity (Nunn et al., 2005; Lindenfors et al., 2007; Morand, 2015). The complex life cycles involving intermediate and definitive hosts means that parasites can only survive in ecosystems where the proper climate and host organisms all come together in the same locality.

Aside from exploring the variations in parasite taxonomic diversity across the empire we can also look at how this has changed through time. With the comparative data gathered here we can broadly analyse changes from the Neolithic period through the Medieval period.

6.1 Temporal Changes – Neolithic Through Medieval

Similar to the point made by Keay (2001), that the Pre-Roman period is important in providing the context for understanding cultural variation in the Roman Empire, the Pre-

Roman and Post-Roman disease-scapes are essential to understanding the presence of disease in the Roman Empire, particularly in teasing apart potential causal factors or determinants of disease. Without an idea of the infectious diseases in a region prior to the time period of interest it is impossible to tell if environmental and sociocultural changes that occurred at that time are responsible for changes in infectious disease transmission; additional comparison with later time periods can also help tease this apart.

Due to the imprecision of many dates for samples studied from the Roman period (many cannot be determined more specifically than a range of two centuries or even more broadly as Roman in general), I have made little mention of changes throughout the Roman period. Nevertheless, we may expect that there were changes in patterns and rates of infection throughout the Roman period as a result of population growth, further adoption of Roman cultural practices in the frontiers, the instability brought about by droughts, regional conflicts, and so on. Further samples and more precise dating may allow us to explore this in the future.

6.1.1 Whipworm and Roundworm

The presence of whipworm and roundworm is quite consistent from the Neolithic period through the Medieval period. These parasites are consistently the most frequently identified intestinal parasite eggs in archaeological samples in these time periods. This exemplifies the ability of these two helminths to infect humans in a range of ecological niches and through varying cultural and social practices. Though prevalence of infection may have changed over time as a result of changing social practices and climate, these intricacies cannot always be established with the current state of palaeoparasitological data as a result of differences in the numbers of sites and locations of sites studied in different time periods, and the lack of knowledge about preservation dynamics of parasite eggs.

When we look at the proportion of sites where roundworm and whipworm were found in the different periods this becomes more clear. Roundworm was found at 40 out of 62 (64.5%) sites in the Roman period, and whipworm was found at 38 out of 62 (61.3%). In the Medieval period, roundworm was found at 41 out of 55 (74.5%) sites studied, and whipworm at 37 out of 55 (67.3%) sites studied. Roundworm is less common in the Pre-Roman period, being found at only 14 out of 46 (30.4%) sites studied, while whipworm is still fairly common being found at 25 out of 46 (54.3%) sites studied. The only significant difference is between the frequency of sites in the pre-Roman and Roman period with *Ascaris* sp. (X^2=12.2693, p=0.0005) (Figure 59). To fairly calculate proportions combining previously published literature and results from this dissertation I have added in the sites that were studied as part of this research; however, I have not counted sites where no parasites were found because

these types of sites are generally invisible in the published record and it is unclear if parasites were not present in the past or just have not preserved. This is the same for the remaining taxa discussed in this section.

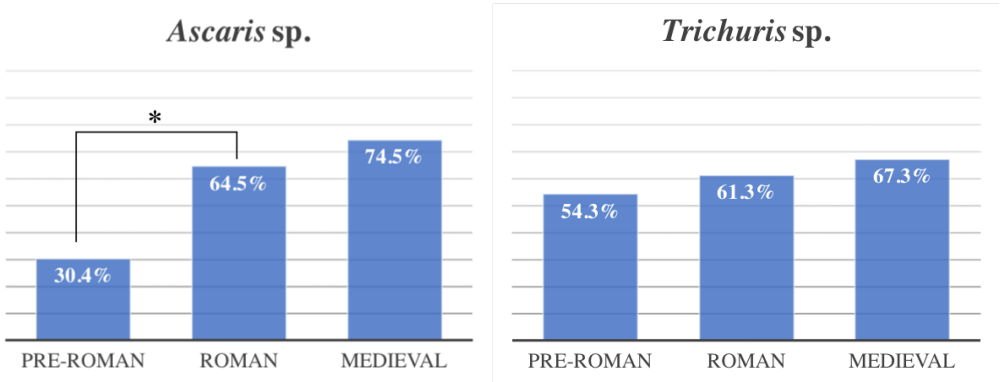

Figure 59: Frequency of positive sites for *Ascaris* sp. and *Trichuris* sp. in different time periods. Significant differences are marked by an asterisk.

Whipworm and roundworm are found spread across areas of Europe and the Middle East that were once part of the Roman Empire, before, during, and after the Roman period (Figure 60 and Figure 61). In the Pre-Roman period the lack of latrines and wider sanitation infrastructure was likely a strong determinant for the presence of soil-transmitted helminths. This was exemplified at the Neolithic site of Çatalhöyük, Turkey (Ledger et al., 2019b; see Appendix A.2) and the Bronze Age settlement of Must Farm, UK (Ledger et al, 2019a; see Appendix A.1). In both of these sites faecal material was dumped in and around living spaces. In the case of the settlement at Must Farm this resulted in general contamination of water around the settlement with parasite eggs as well as recovery of numerous eggs in human faeces. At Çatalhöyük, the presence of rubbish areas near houses that contained human faecal material meant that eggs could be easily transmitted around living spaces resulting in infections in the community.

It is interesting that the proportion of sites that had preserved roundworm eggs was lower in the Pre-Roman period compared to the Medieval and Roman periods yet whipworm was still found at nearly 50% of sites. One possible explanation for this is the number of lakeside settlements that have been studied in the Neolithic period and a few in the Bronze Age. The lakeside settlements that have been studied from the Neolithic period include those from Germany studied by Le Bailly (2005), Chalain and Clairvaux in France (Bouchet, 1997; Bouchet et al., 1995a; Dommelier et al., 1998; Dommelier-Espejo, 2001), Arbon and Parkhaus-Opéra, Zürich in Switzerland (Maicher et al., 2019), and La Draga in Spain (Le Bailly et al., 2003a; Maicher et al., 2017). In the Bronze Age, lakeside or freshwater settlements have been studied from Bourget Lake (Le Bailly and Bouchet, 2010; Le Bailly

and Bouchet, 2013) and Must Farm (Ledger et al., 2019a; Appendix A1). Very few of these sites have roundworm present while most have whipworm, and in general they have the highest level of taxonomic diversity seen in all time periods. Modern epidemiological studies on geographical distribution of soil-transmitted helminths show that climate and environmental factors (e.g. soil pH, rainfall, temperature, elevation, soil type) affect these three parasites differently (Spindler, 1929; Pullan and Brooker, 2012; Pullan et al., 2014; Wardell et al., 2017). Despite these environmental factors that may differentially affect sites studied in different time periods, with increasing urbanisation, population sizes, and resultant waste production we may expect that roundworm prevalence did truly increase in the Roman period.

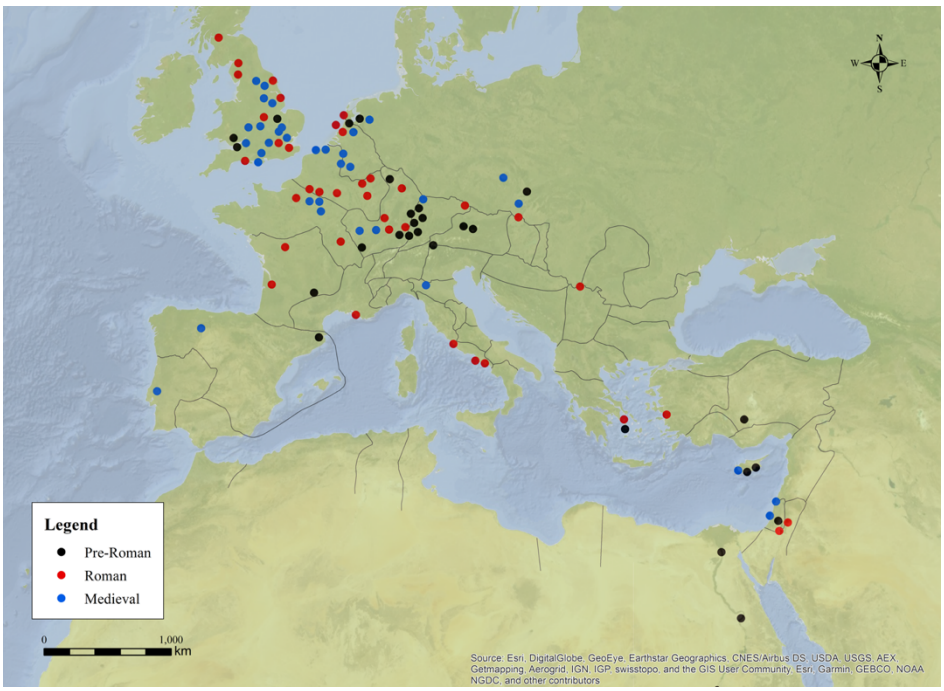

Figure 60: Distribution of whipworm (*Trichuris* sp.) before, during, and after the Roman period. Roman provincial boundaries (117 CE) are from the Digital Atlas of Roman and Medieval Civilizations, Harvard University.

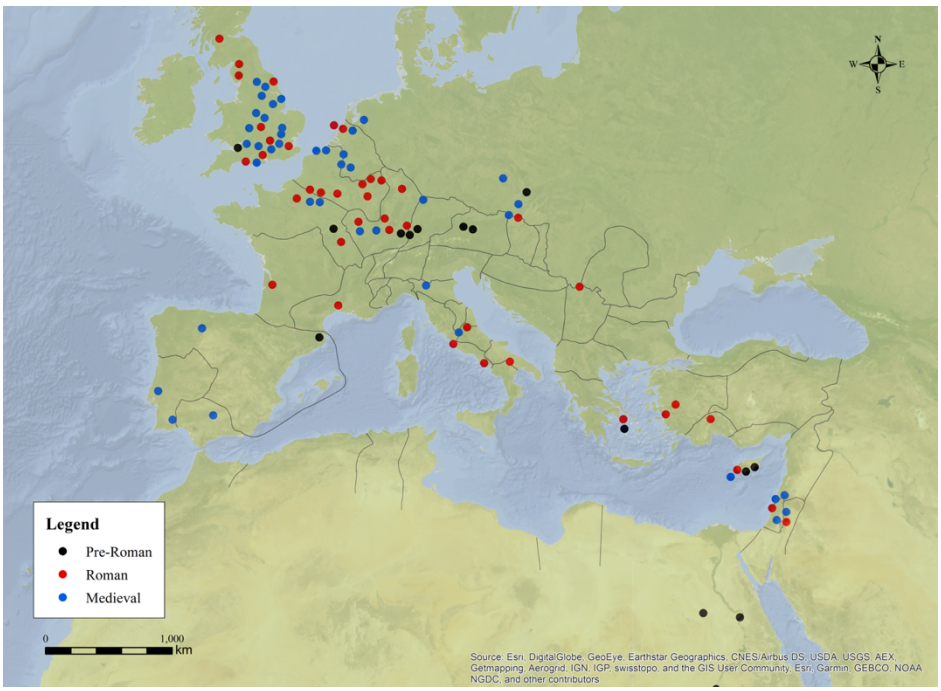

Figure 61: Distribution of roundworm (*Ascaris* sp.) before, during, and after the Roman period. Roman provincial boundaries (117 CE) are from the Digital Atlas of Roman and Medieval Civilizations, Harvard University.

Despite more advanced sanitation infrastructure and widespread construction of latrines in the Roman period, these soil-transmitted helminths are still found in sites across the empire. As has been discussed in each regional focus (see 5.1, 5.2, 5.3, and 5.4), there were numerous other pathways for the movement of helminth eggs around living spaces in Roman communities that may have combined to contribute to a general presence of roundworm and whipworm infections in Roman populations. As has been noted in modern epidemiological studies, latrines and sewers alone are not effective at stopping the transmission of soil-transmitted helminths and there are many other social, cultural, economic, and environmental factors involved in their transmission in communities (Mascarini-Serra, 2011; Schmidlin et al., 2013; Strunz et al., 2014; Worrell et al., 2016; Oswald et al., 2017). Cultural practices that may have contributed to transmission include sharing large latrines, preference for chamber pots especially amongst the elite, and lack of general hand hygiene. These lead to environmental conditions that would have aided in the transmission of these parasites. The emptying of chamber pots in streets, dumping human waste in the street, use of open cesspits without traps or covers, continual defecation in public spaces, and location of latrines in or near kitchens would have resulted in faecal contamination of towns. Economic factors contributing to spread of soil-transmitted helminths include the lack of access to private latrines for everyone and the reuse of human faeces as fertilizer. While the categories of environmental, cultural, and economic can be overlapping it is clear that there are numerous

intricate factors at play in these communities that would have allowed for the spread of faecal-oral pathogens.

Sanitation conditions are not thought to have drastically improved in the Medieval period, if anything they may have gotten worse, and this is likely the reason for the continual presence of roundworm and whipworm at many sites (Mitchell, 2015a). Inadequate disposal of human waste and lack of clean water were likely major factors involved in the continuity of roundworm and whipworm infections. Sanitation infrastructure does not continue to improve in the Medieval period. Many private latrines could still be found with cesspits, and chamber pots were still widely used (Sabine, 1934; Mitchell, 2015a; Taylor, 2015). Flushing latrines were less common while latrines with simple pits or cesspits were more standard. Access to clean water may have decreased as Roman aqueducts fell out of use and underground sewers in many cities were not adequately repaired (Taylor, 2015).

The villa at Vacone offered the opportunity to study intestinal parasites in the Roman period and the subsequent period of Lombard occupation in Italy and has shown possible evidence for the continuation of roundworm infection at the site (see results in 4.3.8 and 4.4.2). The Lombards were a Germanic tribe that migrated from Pannonia and more northern climates into central Italy around 568 CE (Barbiera, 2005) (see Figure 62). This is the first study of parasite infection in Lombard period individuals from Italy. The presence of roundworm eggs in the pelvis of Lombard period individuals from the villa hints that roundworm may have continued to infect people living in Italy after the fall of the Roman Empire, and after the migration of Lombard individuals. However, due to the presence of roundworm eggs in control samples further individuals need to be studied to distinguish true infections from general contamination of the site with roundworm eggs. The only other evidence for parasites from Italy comes from later in the Medieval period (10^{th}–11^{th} c. CE). From Piazza Garibaldi in Parma multiple species were found in cesspit sediment, namely *Taenia* or *Echinococcus* sp., and whipworm (*Trichuris trichiura*) (Bosi et al., 2011; Florenzano et al., 2012). From other pits that were more likely to contain animal faecal material and thus animal parasites, other species found were *Capillaria* sp., *Dicrocoelium* sp., and fish tapeworm (*Diphyllobothrium* sp.). Analysis of additional Lombard period samples and Medieval samples from Italy will allow us to determine if there was continuity between the Roman and Lombard period or if perhaps Lombard populations from Northern Europe introduced new parasites into communities in Italy through migration with parasites or changing cultural practices.

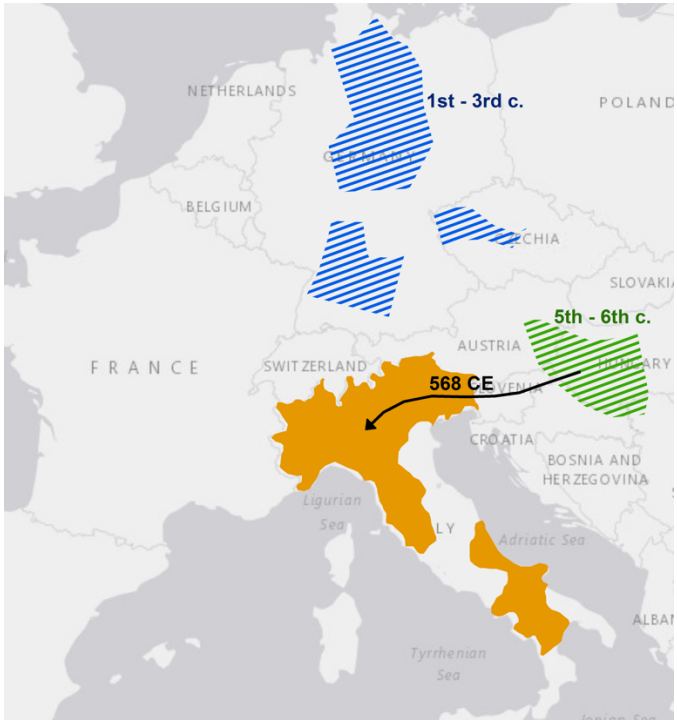

Figure 62: Overview of Lombard migrations into Italy. Proposed areas occupied by the Lombards and path of movement into Italy in the 6th c. CE (adapted from Vai et al., 2015).

The Anglo-Saxon period samples studied from Hatherdene Close also offered the chance to investigate how parasite infection may have changed directly after the Roman period, this time in Britain. Out of the 92 individuals studied, a single parasite egg was found in the pelvic soil of three individuals. One had an egg of *Ascaris* sp., one an egg of *Dicrocoelium dendriticum*, and one an egg of *Trichuris trichiura*. Unfortunately, none of these offer convincing evidence for infection despite the absence of eggs in the control samples. It is possible that the soil in the cemetery was contaminated with parasite eggs and these could have found their way into the burial contexts in very low levels.

The lack of evidence for parasitic infection in the individuals at Hatherdene Close is notable based on the widescale presence in other Medieval sites in Britain. It is possible that a rural living environment, with a smaller population size compared to overcrowded urban centres, may have decreased transmission and spread of these parasites even if they were present in the environment. Though roundworm and whipworm can be maintained in small populations, their prevalence does tend to increase with overcrowding leading to poor sanitation and hygiene in modern populations (Kan et al., 1989; Crompton and Savioli, 1993; Phiri et al., 2000; Pullan and Brooker, 2012).

However, in combination with previously published literature it generally does still appear that roundworm and whipworm remained the most common intestinal parasites infecting people in the Medieval period in Europe. It is possible that roundworm increased in

frequency in the Roman period compared to the Pre-Roman period though this may also be a reflection of the underlying ecology of the sites studied.

6.1.2 Protozoa

None of the sites studied as part of this dissertation provided new evidence for intestinal protozoa in the Roman Empire. Currently the only evidence for *Giardia* in the Roman period comes from a communal latrine at the site of Sagalassos in Turkey (Williams et al., 2017). *Entamoeba histolytica* has been found more widely, at six sites; one in Italy, one in Belgium, three in France, and one in Israel. There is no earlier evidence for *Giardia* from sites studied in the Pre-Roman period, and it then becomes more common in the Medieval period being found at five different sites. *Entamoeba* is found more consistently through all time periods, it has been found at four Pre-Roman sites, six Roman sites, and six sites in the Medieval period. There is no obvious geographic clustering of these protozoan species though it should be noted that most of the samples that have been tested are from central Europe, with very few that I am aware of done on British sites. Similarly, the Mediterranean region is understudied compared to northern Europe more generally.

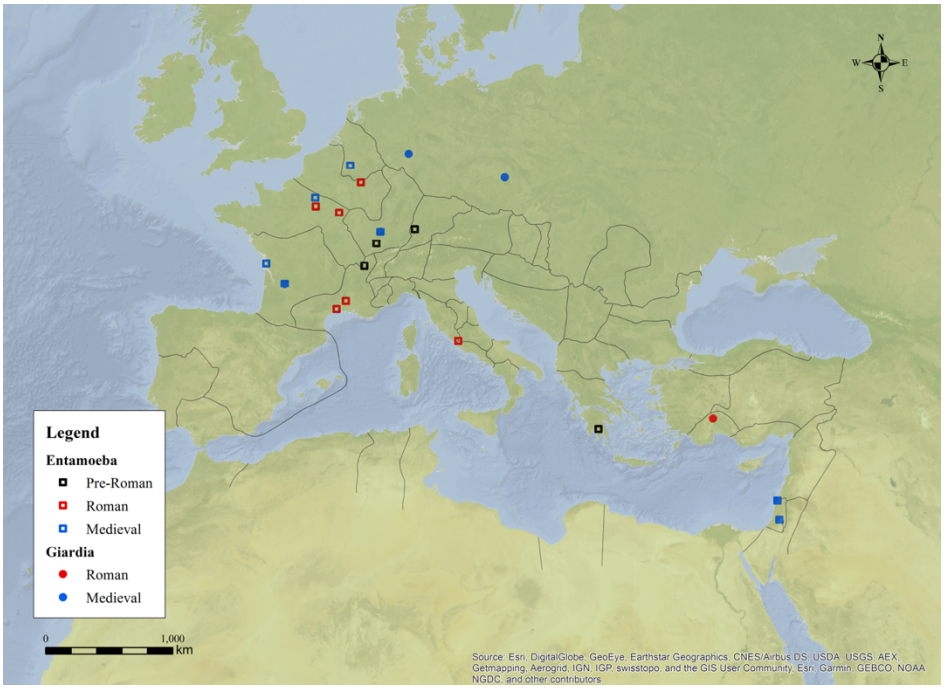

Figure 63: Distribution of *Giardia duodenalis* and *Entamoeba histolytica* before, during, and after the Roman period. Roman provincial boundaries (117 CE) are from the Digital Atlas of Roman and Medieval Civilizations, Harvard University.

6.1.3 Liver flukes

Liver flukes including *Fasciola* sp. and *Dicrocoelium* sp., both primarily zoonotic parasites from sheep, have been found in all time periods in regions that were once a part of the Roman Empire. However, looking at the distribution of them it appears that they are more common in northern Europe with less evidence for them in the Mediterranean and Middle East (Figure 64 and Figure 65), and that this is a pattern seen across all time periods. This becomes most evident when compared to the widespread distribution of sanitation related parasites such as whipworm (Figure 60), roundworm (Figure 61), and gastrointestinal protozoa (Figure 63).

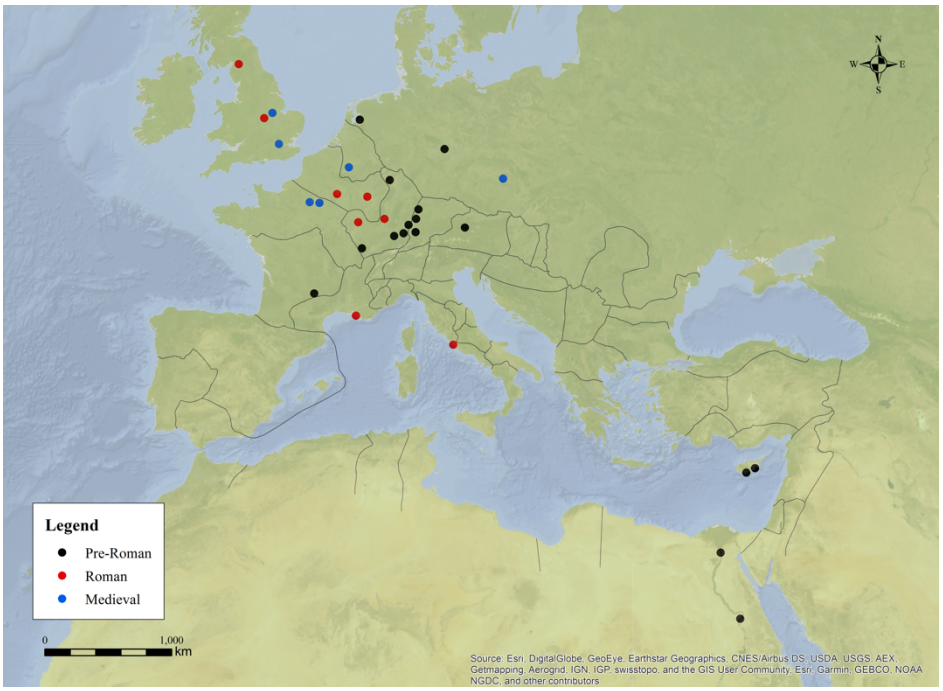

Figure 64: Distribution of *Fasciola* liver fluke before, during, and after the Roman period. Roman provincial boundaries (117 CE) are from the Digital Atlas of Roman and Medieval Civilizations, Harvard University.

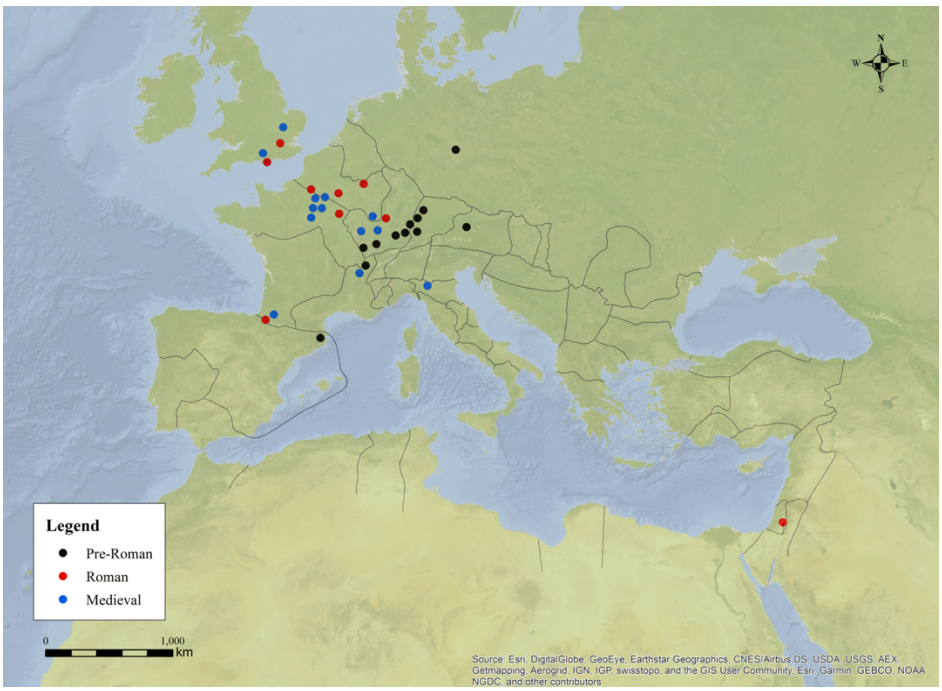

Figure 65: Distribution of the lancet liver fluke (*Dicrocoelium* sp.) before, during, and after the Roman period. Roman provincial boundaries (117 CE) are from the Digital Atlas of Roman and Medieval Civilizations, Harvard University.

In the Roman period both *Dicrocoelium* sp. and *Fasciola* sp. have been found at 8 out of 62 (12.9%) sites, most of which are in Britain and eastern France. In the Pre-Roman period *Dicrocoelium* sp. eggs have been found at 13 out of 46 (28.2%) sites and *Fasciola* sp. at 18 out of 46 (39.1%) sites. In the Medieval period *Dicrocoelium* sp. eggs have been found at 12 out of 55 (21.8%) sites and *Fasciola* sp. at 6 out of 55 (10.9%) sites. Therefore, based on current evidence the proportion of sites where liver fluke eggs were found decreased in the Roman period in comparison to previous Neolithic, Bronze Age, and Iron Age sites, and this is a significant difference (X^2=3.9763, p=0.0461 for *Dicrocoelium* and X^2=9.9378, p=0.0016 for *Fasciola* sp.). Then *Dicrocoelium* increased in the Medieval period and *Fasciola* eggs were still found at about 10% of sites (Figure 66).

Today, the regions of Europe with the highest seasonal risk for *Fasciola* transmission are still in northern Europe, with lower transmission in the Mediterranean region (Caminade et al., 2015). Thus, ecological conditions appear to be a very strong determinant for the presence of *Fasciola* in past and present communities.

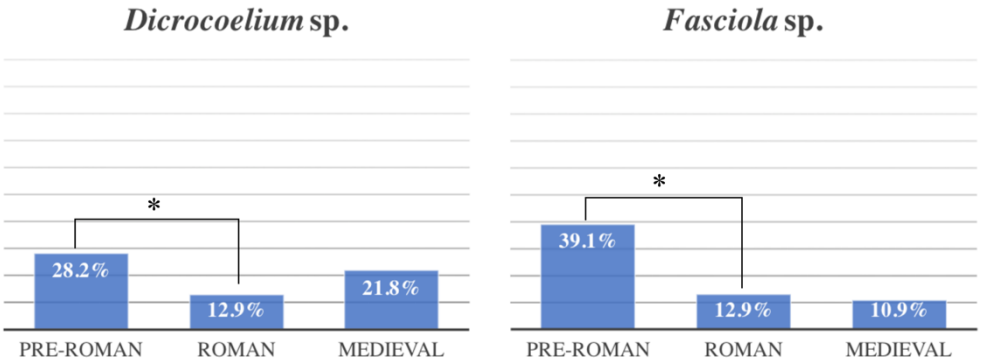

Figure 66: Frequency of positive sites for *Dicrocoelium* sp. and *Fasciola* sp. in different time periods. Significant differences are marked by an asterisk.

The lower rates of liver flukes in the Roman period may be a reflection of the overall decreased reliance on sheep in the Roman period with a general preference for pig and cattle as food sources and working animals (King, 1999). Sheep are common hosts for *Dicrocoelium* and *Fasciola* liver flukes, though cattle can carry the parasites as well. This was not necessarily the case in every area of the Roman Empire and as discussed previously there are some areas, especially rural sites in Britain and areas in northeastern France, where sheep were still relied upon. This may explain the concentration of liver flukes in this area during the Roman period if this practice carried through from earlier Iron Age traditions. Also in these areas humans may have lived closer to water sources where lymnaeid snails (necessary intermediate hosts) are found with sheep and cattle grazing nearby allowing for completion of the parasite life cycle (Mas-Coma et al., 2018).

This is a pattern that has also been noted by Le Bailly and Bouchet (2010) in regards to *Dicrocoelium* presence in Europe during Antiquity. If faeces from infected sheep were used as fertilizer for herbs or other vegetables, cases of pseudoparasitism could result from ingestion of *Dicrocoelium* sp. eggs or true infection if infected ants—the necessary intermediate host, were accidentally eaten with unwashed produce. Similarly in settlements of northeastern France and Britain located near water, ingestion of aquatic plants contaminated with sheep faeces could result in infections with *Fasciola hepatica*. For both types of liver fluke, ingestion of sheep liver or liver of other infected animals could result in passing of eggs in human faeces which could then be found in archaeological samples.

One additional explanation for the lower percentage of positive sites in the Roman period is urbanisation, especially given that many sites studied in the Roman period so far have been larger towns or cities. In these urban areas people were less likely to come into contact with herded animals like sheep or eat aquatic plants such as watercress and dandelion directly from these areas which can have encysted *Fasciola* larvae from sheep faeces. In more

urban centres where people get water from fountains the main risk may have come from buying aquatic plants/vegetables leading to potentially lower rates of infection in larger towns.

6.1.4 Beef and Pork Tapeworm

Taenia spp. tapeworms (beef and pork tapeworms) are found in all time periods. There are very few cases where it has been possible to distinguish between *Taenia saginata* and *Taenia solium* in archaeological settings. This is because the eggs of the two species look very similar morphologically and the most reliable way to distinguish between the two species is using ancient DNA analysis which is time consuming and costly. Although pork is regarded as the meat eaten in a typical Roman elite diet, beef was also fairly common and so it is definitely possible that both species were infecting people in the Roman period. *Taenia* tapeworms have been found at 7 out of 62 (11.3%) sites studied in the Roman Empire. They have been found in more samples from the Pre-Roman period at 14 out of 46 (30.4%) sites, and in more samples from the Medieval period, 11 out of 55 (20%) sites (Figure 67). However, there is only a significant difference between the pre-Roman and Roman period (X^2=6.1790, p=0.0129). Similar to liver flukes these sites are concentrated in areas north of the Mediterranean and in Israel and Egypt (Figure 68).

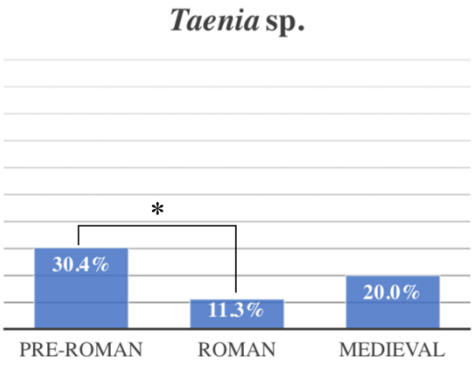

Figure 67: Frequency of positive sites for *Taenia* sp. in different time periods. Significant differences are marked by an asterisk.

The lower proportion of positive samples for *Taenia* tapeworms in the Roman period may reflect a spread of dietary preferences for cooked meat in the Roman period. As has been discussed in the section on *Asia* and the eastern Mediterranean (5.2) eating raw meat was discussed in Roman texts as a repulsive practice of uncivilized people and barbarians in the northern reaches of the empire (Garnsey, 1999b; Chandezon, 2015). These texts give us the opinion of elite Romans, likely from the Mediterranean region, and though their opinions

cannot necessarily be trusted as to how individuals living in the northern provinces of the empire may have cooked their food, they do highlight a distaste for raw meat. In order to kill *Taenia* larvae in raw pork or beef, pork needs to be heated to a temperature of 65ºC and beef to 56 ºC (Wittner et al., 2011). Leaving beef in brine with a concentration of 25% has been reported to kill larvae in 5–6 days. In general, traditional salting and smoking recipes for pork and beef are not always effective at killing larvae (Rodriguez-Canul, 2002; Rivera-Guerrero, 2004). Though there is evidence for smoking meat (Cool, 2006:89) and recipes for salted meat (*Apicius,* 1.7) in the Roman Empire it is possible that most meat was eaten cooked.

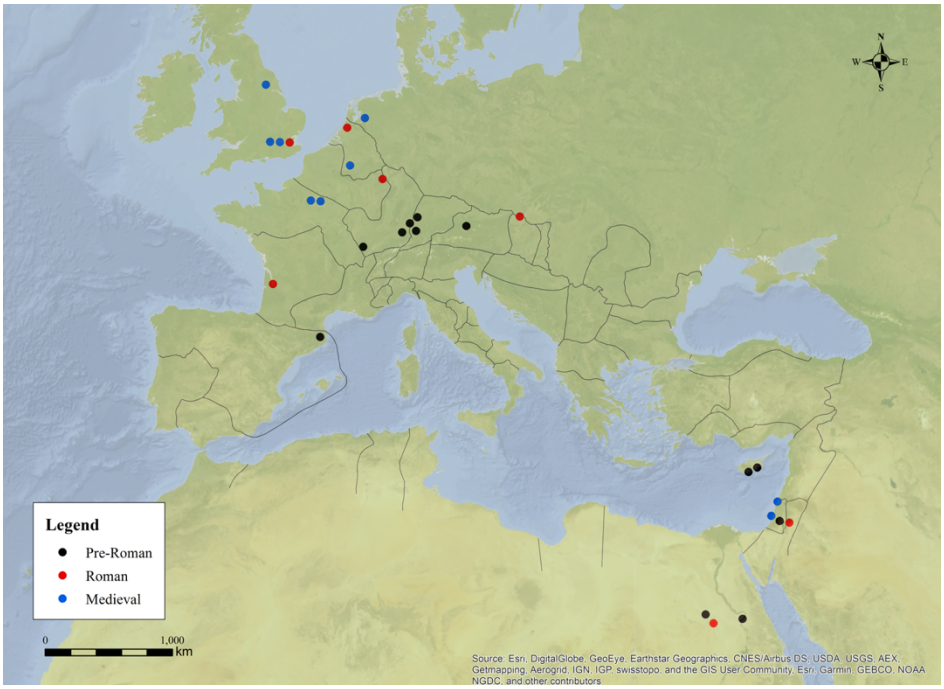

Figure 68: Distribution of beef/pork tapeworm (*Taenia* sp.) before, during, and after the Roman period. Roman provincial boundaries (117 CE) are from the Digital Atlas of Roman and Medieval Civilizations, Harvard University.

6.1.5 Fish tapeworm

When we look at the distribution of fish tapeworm from the Neolithic period through the Medieval period it appears that fish tapeworm is more common in the Pre-Roman period and Medieval period than it is during the Roman period. In other words, fish tapeworm was less common in the Roman period than earlier or later time periods. It was found at 6 out of 62 (9.7%) sites, when the newly studied sites in this dissertation are added not counting Arlon twice. In the Pre-Roman period it was found at 16 out of 46 (34.8%) sites studied from the Neolithic, Bronze Age, and Iron Age. In the Medieval period it was found at 9 out of 55 (16.4%) sites studied (Figure 69). The only significant difference is between the pre-Roman and Roman period (X^2=10.2608, p=0.0014).

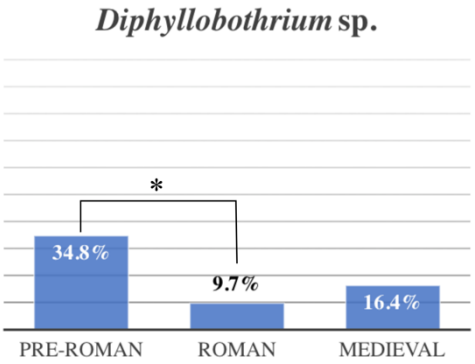

Figure 69: Frequency of positive sites for *Diphyllobothrium* sp. in different time periods. Significant differences are marked by an asterisk.

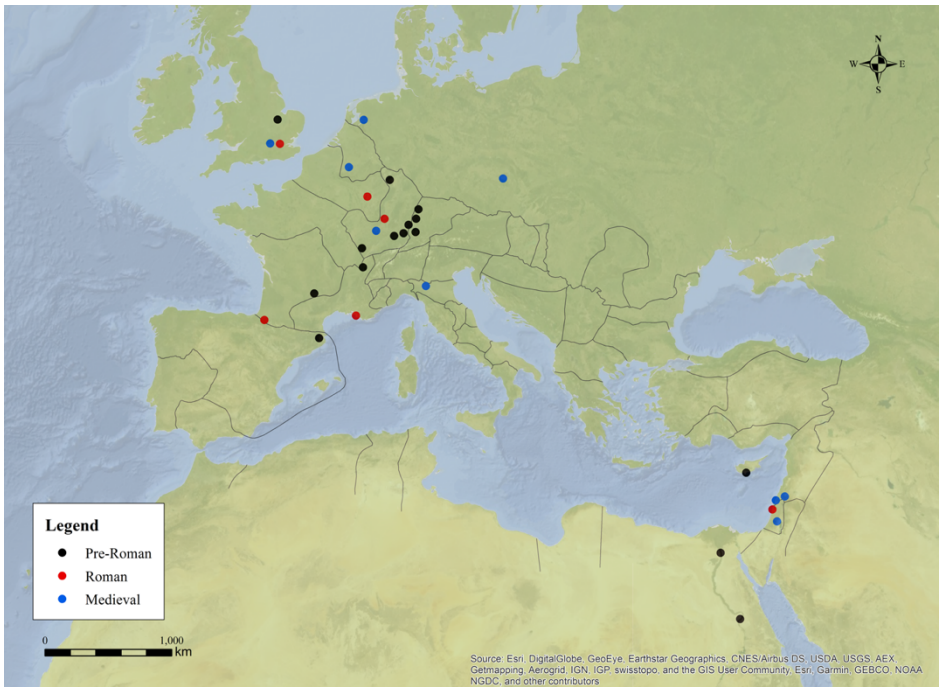

Figure 70: Distribution of fish tapeworm (*Diphyllobothrium* sp.) before, during, and after the Roman period. Roman provincial boundaries (117 CE) are from the Digital Atlas of Roman and Medieval Civilizations, Harvard University.

Looking at the proportions it becomes clear that fish tapeworm was most common in the Pre-Roman period, particularly in the Neolithic period, and then decreased in the Roman period and increased slightly again in the Medieval period. The increase in fish tapeworm infection from the Roman period to the Medieval period has been noted by other authors and is expected to be a result of increased fish consumption as a result of the spread of Christianity in Europe, which imposes restrictions on meat consumption on specific days (Mitchell, 2015a). However, as mentioned previously, in areas of the empire such as Britain zooarchaeological evidence has shown an increase in fish consumption in the Roman period compared to the Iron Age. The only evidence for Pre-Roman fish tapeworm in Britain comes from the Late Bronze Age settlement at Must Farm. Due to the lack of studies on Iron Age

sites it is difficult to know what was happening in other areas of Europe immediately prior to the Roman period and the pattern of lower rates of fish tapeworm may have already started in the Iron Age.

As I have mentioned in previous sections, the presence of fish tapeworm in the Roman period in northern provinces including *Britannia*, *Belgica*, *Germania Superior*, *Aquitania*, and *Lugdunensis* may be a reflection of fish consumption in the Roman period in the form of inadequately cooked fresh fish and preserved fish. The data from preceding periods shows that fish tapeworm was able to survive in many regions of the empire though it is not found there in the Roman period. This may be a result of preferences for certain types of fish and preparation of fish. Though both freshwater and marine fish were sold and consumed in the Roman Empire there appears to be a preference for marine fish, especially amongst the elite. Based on Diocletian's *Edict on Maximum Prices* we know that marine fish were generally more expensive than freshwater fish (Marzano, 2018). Fish was very popular and when it was eaten fresh, it was often eaten cooked (Giacosa, 1992:122). It has been thought that most fish in the diet of the Romans came from the sea and that river fish were not as common (Curtis, 1991:14). In the sewers at Herculaneum for example, remains of a wide variety of plants were found alongside animal bones that were dominated by coastal fish and shellfish (Robinson and Rowan, 2015). This pattern is likely different than in the northern provinces where freshwater lakes and rivers were more common and would have made acquisition of freshwater fish much easier than marine fish.

Long distance trade of fish and fish products did occur. For example, at Sagalassos in Turkey aDNA analysis of fish remains have identified a species of fish that must have come from the Nile valley (Arndt et al., 2003). In most cases in order for long distance trade of fish to occur without spoiling, the fish would have needed to be preserved. This would have been in the form of popular Roman fish dishes including salted fish and fish sauce. Though the terminology has been debated salted fish is called *salsamenta* and fish sauce can be either *garum*, *liquamen,* or *muria,* while the residue or sediment from making the sauce was called *allec* (Grainger, 2013; Grainger, 2018). It is thought that preserved fish in its various forms was accessible to people of varying socioeconomic statuses and while there were certain sought after varieties it was widely made and consumed across the empire (Marzano, 2018).

The practice of processing fish by salting appears to have spread from the eastern Mediterranean prior to the Roman period and was well established across the Mediterranean by the 5th c. BCE (Mylona, 2018). Salting workshops are found along the Mediterranean coast and fish sauce (*garum, liquamen, muria*) was produced in high quantities in the Spanish and

African provinces and transported around the empire (Garnsey, 1999a:16; Van Neer et al., 2010). There is also evidence for local production of fish sauce, for example in Britain (see 5.4), Gaul and Belgium (see 5.3). For a consistent supply of fish, farms were set up along the coasts where a range of fish could be farmed to be sold in markets, and also used to produce *garum* and *salsamenta.* Kron (2015) notes that in the early Imperial period 82 fish farms had already been established along the coasts of Italy.

Various fish were used to make fish sauce and these would have varied by region. A recipe from the 3rd c. CE by Gargilius Martialis, now considered to be a Medieval gloss, specifies fatty fish such as sardines should be used to make the sauce (Giacosa, 1992; Grainger, 2013). It specifies that there should be a layer of salt on top of the fish that is two fingers high and that it should sit for seven days in the sun then be mixed daily for twenty days (Gargilius Martialis, *Medicinae ex holeribus et pomis*, 62; Grainger, 2018). There are two additional recipes in the *Geoponica*, a Byzantine text, that was a 10th-c. farming manual that preserved material from the entire Roman period. One of these recipes states that the mixture should be pickled for 2–3 months (Grainger, 2013). Pliny wrote that any fish could be used but mackerel was most common (Curtis, 1991:14). Lower quality garum may have been made for slaves using leftover parts of fish that would not have been eaten by the household (Giacosa, 1992:28) and local varieties would have likely relied on fish that could be caught nearby. Fish sauce produced in the Mediterranean mainly used sardines, sardinella, anchovies, and sea breams, whereas mackerel and scad were used for salted fish (*salsamenta*) (Van Neer et al., 2010).

Despite this widescale evidence for fish consumption, including preserved fish, the rates of fish tapeworm infection do not appear to be very high in the Roman Empire as a whole. The preference for marine fish may be in part responsible for this pattern as the species of *Diphyllobothrium* found in Europe can only use freshwater fish as an intermediate host. There is evidence for freshwater fish consumption, especially as it was a cheaper variety of fish and more commonly found in some of the northern provinces of the empire. However, this freshwater fish was often eaten cooked. Inadequate cooking in some cases may have resulted in cases of fish tapeworm infection.

6.1.6 *Capillaria* and Other Helminths

One other taxa that is found in all time periods is *Capillaria* sp., though it is found more commonly in the Pre-Roman time periods and the Roman period compared to the Medieval period. It has been found at 9 sites in the Pre-Roman period, 8 of which are Neolithic period and one is the Late Bronze Age site of Must Farm. In the Roman period *Capillaria* sp. has

been found at six sites, and in the Medieval period it has only been found at two sites (Figure 71). None of these differences are significant. Similar to other zoonotic parasites such as the liver flukes and *Taenia* tapeworms it is found most commonly in northern Europe in all time periods. Two sites that stand out as farther south than the rest of the sites where it has been found are in northern Italy and Spain, one is Pre-Roman and one is Medieval (Figure 72). In the Roman period all *Capillaria* sp. eggs have been found in sites in *Belgica* and very near to its borders, these sites are in the modern-day countries of France and Germany.

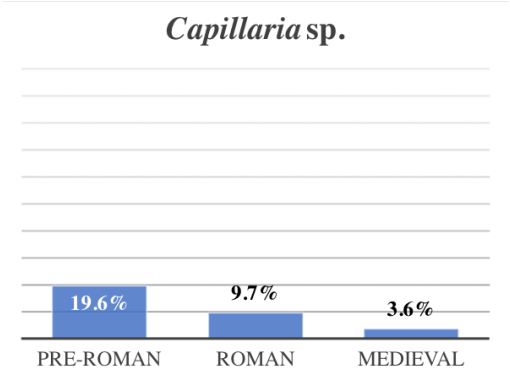

Figure 71: Frequency of positive sites for *Capillaria* sp. in different time periods. Significant differences are marked by an asterisk.

There are over 300 species of capillarids known, with diverse life cycles and in most archaeological settings it has not been possible to identify the species found but eggs are simply identified as *Capillaria* sp. (Borba et al., 2019). *Capillaria* is a common parasite of rodents but can also be acquired from fish consumption, a possible explanation for the presence of this taxa at the Must Farm settlement (Ledger et al., 2019a). Another species known to infect humans is *Capillaria hepatica*, which is acquired from ingestion of infective eggs in the soil. However, an infected individual does not release eggs in the stool as these are found in the liver in the case of true infection. One convincing case of *Capillaria hepatica* infection in the Roman period comes from the site of Amiens in France where *Capillaria hepatica* eggs were seen in an *Echinococcus* cyst of the liver indicating concurrent infection with these two species (Mowlavi et al., 2014).

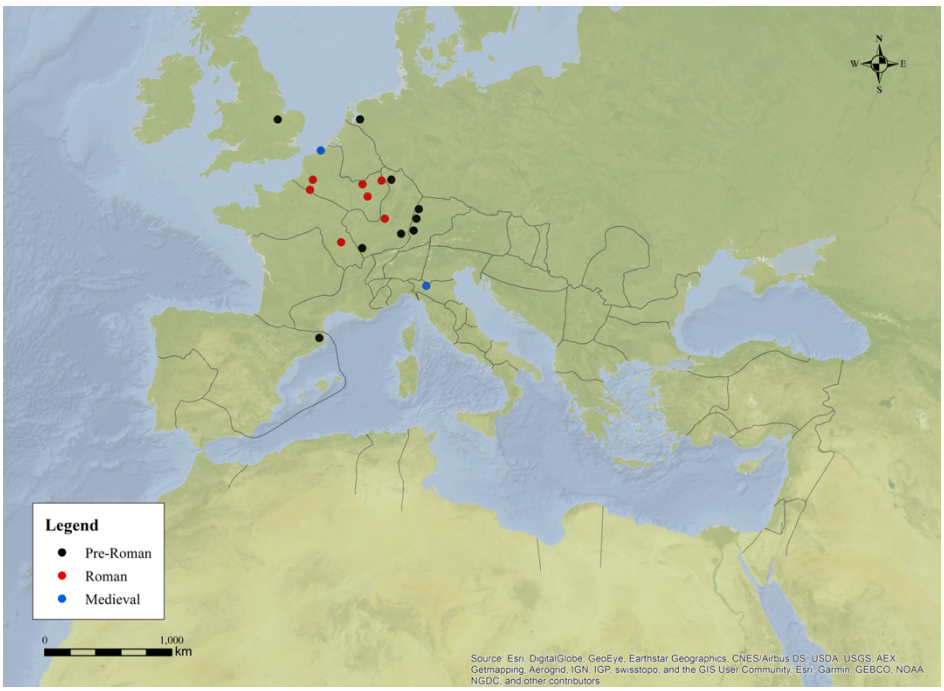

Figure 72: Distribution of *Capillaria* sp. before, during, and after the Roman period. Roman provincial boundaries (117 CE) are from the Digital Atlas of Roman and Medieval Civilizations, Harvard University.

There are some other taxa of parasites that are found in the Pre-Roman period but not in the Roman period such as *Ancylostoma* sp., *Dioctophyma renale*, *Echinostoma* sp., and *Schistosoma* sp. Similarly, there are some parasites found in Egyptian mummies in the Pre-Roman period that are not found in the Roman period such as *Dracunculus medinensis* and filarial worm. However, the additional findings in mummies is due to enhanced preservation of the worms as a result of the mummification process. In general, there is a higher diversity of parasites found in Neolithic sites compared to other time periods. The Bronze Age and Iron Age also have some very taxonomically diverse sites, though far fewer sites have been studied in these periods. In general, a more diverse parasite burden is found in Neolithic communities due to consumption of undercooked food, wild animals, and insects resulting in infections with species that are more rare today such as *Dioctophyma renale* and *Echinostoma* (Le Bailly et al. 2003a; Fugassa et al. 2010; Ledger et al., 2019a). With domestication of animals, beginning around 16,000 ya (Perri et al., 2019), and increasing reliance on farmed animals in the Roman period there appears to have been an increase in parasites from domesticates, such as *Taenia*, *Fasciola*, and *Diphyllobothrium* (Sianto et al. 2009).

In the Medieval period we see the first evidence for *Hymenolepis* sp. and *Strongyloides* sp. neither of which was found at the sites studied in the Pre-Roman or Roman period in regions that were once part of the Roman Empire.

6.2 The Roman Perspective

6.2.1 Roman Understanding of Parasites

Having established the presence of intestinal parasites in many Roman communities, especially soil-transmitted helminths, one can ask the question; what did the Romans themselves understand about these pathogens? While microorganisms were not accepted as the cause for disease until the end of the 19th century, helminths are unlike bacteria and viruses in that they are large enough to be seen and thus we may expect that there was some knowledge about what they were, where they came from, the symptoms they caused, and how they should be treated. There are various sources we can use to get an insight into the Roman perspective on intestinal diseases including medical texts, other historical texts, and depictions in art. There are of course limitations to these sources in that they mainly provide evidence for what a specific group of people understood, and in many cases when we look at Roman texts these are elite individuals often from the Mediterranean. Furthermore, even in the Roman period there were multiple schools of thought in medicine, and thus various understandings of what caused diseases and how they should be treated (Hanson, 2010:520). Due to the limited number of surviving texts, our understanding from these texts is not necessarily a balanced picture of the various opinions that existed at the time. In addition, the individual describing the disease may not have actually interacted with that disease but instead be recounting their knowledge of it gained from someone else, possibly even writing in another time period (Mitchell, 2011). Artistic representations are not always accurate or can be exaggerated, thus they also need to be interpreted with caution.

Prominent Roman medical writers include Cornelius Celsus (26 BCE–50 CE), Scribonius Largus who wrote a collection of prescriptions in 47 CE for the emperor's freedman, Soranus of Ephesus who practiced during the reign of Trajan and Hadrian (98–128 CE), and the physician Galen of Pergamum (129–216 CE). Other informative texts come from Marcus Porcius Cato the Elder (234–149 BCE) whose text *On Agriculture* describes the many principles involved in managing an estate but also provides medical advice for managing healthcare in one's own household, the job of the *pater familias*. Many of these writers described intestinal worms or treatments for worms in their works.

From Celsus we get a description of two types of worms—roundworms and flatworms. He also describes that roundworms "especially trouble children" (*De Med. 4.24.2)*.

"Again, worms also occasionally take possession of the bowel, and these are discharged at one time from the lower bowel, at another more nastily from the mouth: and we observe them sometimes to be flattened, which are the worse, at times to be rounded." (De Med. 4.24.1).

Galen describes three types of intestinal worms (*lumbrici*), *lati*, *teretes*, and *ascarides*. Some believe that *lumbrici teretes* or 'round worms' are roundworm (*Ascaris lumbricoides*), the *ascarides* are smaller round worms possibly *Enterobius vermicularis* (pinworm), and that *lumbrici lati* are tapeworms (Jirsa and Winiwarter, 2010). These three types of worms were also described by Aristotle (Hoeppli, 1956). As we can see there was a general knowledge of the presence of intestinal worms, especially those that are common and visible. Roundworm can grow up to 15–30 cm in the human intestines and thus when expelled would have been very visible. Similarly, tapeworm expels pouches of eggs, proglottids, that are often visible in the stool. Though these common worms were known, it does not appear that all types of intestinal worms found in the Roman period were described. However, some may have been grouped into these three categories and some may have been recognized only in animals.

Galen also describes the origins of intestinal worms in his works. He believed that worms arose in the bowels and were not acquired from outside the body, and that they were formed from the putrefaction and heat or rotting of organic material (Jirsa and Winiwarter, 2010). He also acknowledged that they could be found in other animals. Based on his views that worms did not arise from outside the body, it seems unlikely that he would believe animals could pass parasites to humans. Similarly, the risk of acquiring parasites from one's external environment directly does not fit with the idea that they are generated within the body.

Galen also recognizes that worms are particularly prevalent in children but links this to the fact that worms need a lot of heat to be generated. In alignment with theories of the four humours and necessary balance of warm and cold at the time, it was believed that in childhood blood dominates over other humours and thus children are warmer which makes them more susceptible to worms (Jirsa and Winiwarter, 2010). This fits with what we know about the transmission of gastrointestinal helminths today, children are a high risk group for infection. More specifically those between 5–15 years, or school-aged children in modern populations, are more often infected in endemic communities compared to older or younger individuals (Bundy et al., 1987; Crompton and Nesheim, 2002). While there is ample evidence for higher intensities of infection in children there are fewer studies directly looking at factors responsible for infection in this group. Some proposed factors include outdoor play

in areas with infected soil, open defecation, maternal education, and hand hygiene (Wang et al., 2012; Krause et al., 2015; Pabalan et al., 2018).

Galen, who was a follower of Hippocrates often citing his work, spread the humoral theory. Using the four humors (blood, phlegm, yellow bile, and black bile) from the *Hippocratic corpus* he proposed that health was achieved through a necessary balance of these bodily fluids and the four qualities (hot or cold and wet or dry). Though proposed at the time it is unclear to what extent this theory was accepted during Galen's life. Certainly, there were other medical sects practising in the Roman period who were not followers of Hippocrates such as the Methodists, Empiricists, Erasistrateans, and Pneumatists (Nutton, 2004; Nutton, 2005). The ideas of these other groups in regards to the origins and treatments for intestinal worms are unclear, though we can imagine that there were a variety of understandings and ideas about how they should be prevented and treated, if at all.

6.2.2 Medical Treatments

There are numerous treatments for intestinal worms in the works of medical writers and others from the Roman period. Celsus (25 BCE–50 CE) mentions treatments for roundworms and flatworms including a decoction of lupins or mulberry bark with hyssop, or a vinegar of pepper. He also suggests eating garlic and vomiting, followed by drinking a mix of boiled pomegranate roots with soda the following day. Additionally, to treat roundworms he suggests pounded seeds of nettles, cumin, cabbage, or mint in water. He also suggests a decoction of wormwood, hyssop, or cress seeds in vinegar (*De Med.* 4.24.1–2).

Galen (129–210 CE), as one of the most prolific medical writers of his time mentions numerous treatments for intestinal worms in his works. Many of these are plant based such as mint, cardamom, myrrh, and cumin as well as artemisia specifically for roundworms (Jirsa and Winiwarter, 2010). Most of the treatments described by Galen are very aromatic and bitter and were thought to have drying effects. These properties were important as he believed that worms were spontaneously formed by putrefaction and heat in particularly moist environments (Jirsa and Winiwarter, 2010). While some of these herbal remedies have been shown to be effective at treating certain types of parasites, such as artemisia (Tagboto and Townson, 2001; Cheraghipour et al., 2018), this relies on the dosage or the quantity of the herbal remedy taken, and we do not know what this would have been in the Roman period.

Medical texts from prominent Roman physicians, such as Celsus and Galen, give us a view of medical beliefs at the time from the perspective of high status physicians. Though doctors (*medici* or *iatroi*) were present in many Roman cities and one could enter a system of

informal training to become a physician, the profession was not regulated and anyone could assert themselves as a doctor. Rather the qualifications of doctors appear to have been based on who they trained under (Harris, 2016:32). Medical treatment would have also fallen under the responsibility of the head of the household, the *pater familias*, who would treat the day-to-day ailments of his household. Aside from these documented forms of medicine there would have also been forms of 'popular medicine' adopted by the general public, especially by those who could not afford to hire a doctor. Popular medicine as opposed to what has been called the rational medicine of doctors includes the healing invoked from deities such as Asclepius, healers, drug-sellers, and the numerous herbal cures and remedies that would have been passed between lay people.

Others writing in the Roman period who would not be considered doctors or healers are Cato (234–149 BCE), acting as a *pater familias*, who gives us one insight into what popular medicine or folk medicine suggested for intestinal worms. In his treatise *On Agriculture,* he suggests that for tapeworms or stomach-worms one should crush 30 acid pomegranates and mix them with black wine to set for 30 days (*De Re Rust.* 126). While that does not seem to fall too far from the treatments of Galen or Celsus, he then recommends that the patient needs to climb a square pillar and jump down ten times before going for a walk (*De Re. Rust.* 127.1–3). Pliny, writing in 77 CE, describes a decoction of wild lupins with rue and pepper as a treatment to expel intestinal worms in people under thirty (*Nat. Hist.* 22.74.15).

Many of the suggested remedies for treatment of intestinal worms have not been systematically tested to determine their efficacy in humans. Therefore, the anti-parasitic properties of the treatments suggested by Roman writers to treat intestinal worms is largely unknown. Certain herbal remedies such as artemisia have shown some efficacy in treating parasites though not all types of parasites. Artemisia and it's derivatives have been shown to be effective in treating malaria and schistosomiasis in humans (Dhingra et al., 2000; Ogwang et al., 2012; Munyangi et al., 2018). Experimental studies have also shown that artemisia is effective at killing the cestode *Hymenolepis nana* in mice (Beshay, 2018). Pomegranate, a treatment suggested by Celsus and Cato, has been shown to be effective in reducing oocyst shedding in mice infected with *Cryptosporidium parvum* (Al-Mathal and Alsalem, 2012). In *in vitro* tests, it was also shown to be active against *Entamoeba histolytica* (Calzada et al., 2006); though its effectiveness as a treatment in humans is unknown. This evidence shows that some of the treatments suggested could have treated certain species of parasites though likely not all of them, and there is no experimental evidence for the effectiveness of these

remedies in treating parasites that were found to be ubiquitous in the Roman period such as roundworm and whipworm.

Symptoms from intestinal worm infections are often minor or even asymptomatic. Therefore, in many cases individuals may have not known they were infected until a worm was shed during a bowel movement. An exception would be with cestode infections where proglottids can be seen in the stool during the course of infection. In heavier infections symptoms often include abdominal pain and diarrhoea or constipation. These types of symptoms were likely more commonly treated than the worms themselves and there are countless suggestions for how to deal with general intestinal upset in the works of Galen, Celsus, and many other writers at the time.

6.2.3 Impacts on Health

Average life expectancy at birth in the Roman Empire has been estimated to be 22–25 years and if a child made it to 10 years of age this increased to 36–38 years based on census data from Roman Egypt (Bagnall and Frier, 1994). While these estimates have to be taken as rough estimates due to problems with interpreting the census data and the wide variations in life expectancy that must have existed across the empire (Scheidel, 2001), what we can conclude is that life expectancy was probably more in line with patterns seen in developing countries today. High infant mortality and childhood mortality would have pulled average life expectancy down while endemic infectious disease and periodic epidemics would have resulted in higher mortality than is seen in western countries today. Today one of the leading causes of mortality in children under five is diarrhoea, and this was probably similar in the Roman period. Despite this we know very little about gastrointestinal diseases in past populations due to the difficulties in finding evidence for the presence of these pathogens. In fact, gastrointestinal diseases in general were probably commonplace in the Roman period, and certainly the evidence for intestinal parasites points to their consistent presence in communities across the empire.

Disease in the Roman period has been widely studied from various perspectives, often with the intent of focusing on certain diseases or epidemics. Diseases with high mortality such as malaria and major epidemics, such as the Antonine Plague and the Plague of Cyprian, known from historical accounts have been widely studied. There are full books on malaria (Sallares, 2002) and ancient DNA studies confirming its presence in Roman Italy (Marciniak et al., 2016). Aside from focus on particular diseases, some regions have also been well studied for disease more broadly. For example, Roberts and Cox (2003) provide an account of diseases in Roman Britain known from palaeopathological evidence. Similar work has been

done for Rome (Minozzi et al., 2013; Scheidel, 2013). Other diseases that have been widely studied and recorded in Roman period skeletal samples are vitamin D deficiency (Mays et al., 2018; Lockau et al., 2019; Peacock et al., 2019), tuberculosis and brucellosis (Capasso, 1999; Anderson, 2001; Lewis, 2011; Redfern et al., 2015), and trauma (Redfern and Chamberlain, 2011; Gilmour et al., 2015; Timmins et al., 2017; Gilmour et al., 2019). Other non-specific markers of disease such as cribra orbitalia, porotic hyperostosis, and linear enamel hypoplasia are often recorded on skeletal samples though they cannot be used to determine the presence of specific diseases (Facchini et al., 2004; Cucina et al., 2006; Belcastro et al., 2007; Lewis, 2010; Redfern et al., 2015).

Cribra orbitalia and porotic hyperostosis are often linked to parasitic infection (intestinal worms and other parasites such as malaria) but are also described as resulting from any number of conditions that can cause anaemia. However, there is no reliable experimental evidence linking cribra orbitalia and porotic hyperostosis to anaemia and nutritional deficiency in humans, except for one small study done in rats (Polo-Cerdá et al., 2001). Rather, this discussion relies on knowledge of bone physiology and linking of pathophysiological processes in anaemia with the bony changes commonly visible with these lesions (Stuart-Macadam, 1985, 1987; Wapler et al., 2003; Walker et al., 2009). Studies looking at rates of cribra orbitalia and porotic hyperostosis in Roman period skeletal samples have noted that they are quite frequent in some skeletal samples. From Rimini and Ravenna in Italy cribra orbitalia was found in 56% of all skeletons analysed and porotic hyperostosis in 40% (Facchini et al., 2004). From Vallerano, near Rome, cribra orbitalia was found in 69% of individuals (Cucina et al., 2006). At Lucus Feroniae in Italy 50% had cribra orbitalia (Salvadei et al, 2001). From Poundbury, Dorset in Britain 39% of nonadults had cribra orbitalia (Lewis, 2010). While some of these rates are quite high it seems unlikely that intestinal parasite infections are major contributors even though they are commonly mentioned in the list of contributing diseases. Numerous samples from Roman sites studied as part of this dissertation had no preserved eggs. While it has to be acknowledged that a number of eggs may have not survived since the Roman period it does not appear that everyone in these cities was infected. Furthermore, the most common parasites found, which it could be argued most people were exposed to, were roundworm and whipworm which do not always cause anaemia. Specific parasites such as hookworm, fish tapeworm, and schistosomiasis are known for causing anaemia but these are not the most commonly found parasites in Roman period sites. Roundworm and whipworm can cause anaemia but typically only in cases of high worm burdens and severe nutrient deficiencies. This evidence further exemplifies that there is no reliable skeletal evidence for parasitic infection.

Most of the parasites found in the Roman period samples still infect large numbers of people today. In fact many of them are classified as neglected tropical diseases by the World Health Organization. Soil-transmitted helminths (roundworm and whipworm), taeniasis, and foodborne trematodes including *Fasciola* are all classified as neglected tropical diseases. The goal of the list is to raise awareness and funding for these diseases that still infect large numbers of people but do not receive as much funding or research attention compared to diseases that cause high mortality like malaria and HIV, and that primarily infect people in poorer countries (Stolk et al., 2016). The impacts of these diseases are often quantified using disability adjusted life years (DALYs) rather than mortality because they cause more disability than mortality. DALYs are a sum of the years of life lost due to premature mortality and the years of life lived with disability. The negative health impacts of these diseases have been well studied in modern populations and this can give us an idea of what impacts these same diseases may have had in the Roman period.

It is estimated that around 1.3 billion people today are infected with roundworm or whipworm and this is primarily in subtropical regions of the world (Jourdan et al., 2018). In 2010 it was estimated that roundworm and whipworm alone account for 1.9 million DALYs. Foodborne trematodes account for another 1.9 million and other diseases including *Taenia, Diphyllobothrium,* and protozoa account for 4.7 million (Hotez et al., 2014). What this shows is that even though we would not expect the parasites found in the Roman period to cause high mortality they would have had a measurable impact on the daily life of people infected.

Starting with roundworm and whipworm, the two most common parasites found in the Roman period, common symptoms include abdominal pain, diarrhoea, nausea/vomiting, and in more severe cases they can cause intestinal obstruction and anaemia from nutrient deficiencies. In relatively healthy and well-nourished individuals a few worms would have little impact. However, in children—who are often more likely to be infected, the disease can be more severe. Roundworm and whipworm can cause decreased food intake, malabsorption, and decreased growth in children (Crompton and Nesheim, 2002). High worm burdens in particular cause these nutrient deficiencies leading to impaired cognition and stunting of growth (Bethony et al., 2006). In modern populations this in turn results in children being less likely to go to school or to work which has long-term economic impacts (Crompton and Nesheim, 2002). Children between the ages of 5 and 15 years usually have the heaviest and most frequent infections (Hotez et al., 2008). This appears to be due to a combination of acquired immunity decreasing infections in adulthood and social practices decreasing the chances that an adult is infected compared to a child. This acquired immunity can work to

prevent a worm from establishing an infection, decrease the longevity of that infection, or decrease the harmful effects of a prolonged immune response (Anthony et al., 2007; Artis and Maizels, 2011). For the reasons mentioned above, we may expect a similar pattern in the Roman period though the sample types currently studied do not allow us to differentiate who has infected within a community.

Based on the widescale presence of roundworm and whipworm across the Roman Empire we may expect that infections with these parasites were widespread. The minor symptoms they caused, especially in adults with good nutritional status and low worm burdens, may have been viewed as a regular part of everyday life. Heavier infections, resulting in acute symptoms may have caused more distress and possibly in some causes resulted in people seeking treatment. Though it is expected that a low proportion of the population had heavy worm burdens due to the fact that these parasites are often overdispersed within populations. Cato mentions that his treatment for intestinal worms is only necessary if the worms are troublesome (*De Re Rust. 126*), indicating an understanding that they are not always detrimental to one's health. The number of other minor gastrointestinal diseases present in these communities due to inadequate sanitation measures, that also contributed to the presence of soil-transmitted helminths, may have meant that minor abdominal upsets were quite common and certainly the number of herbal remedies and dietary suggestions for dealing with intestinal upsets by Galen, Celsus, and Pliny indicates they were a common complaint that may have been accepted as a normal part of everyday life.

Protozoan infections such as *Entamoeba* and *Giardia,* though less common, would have had larger impacts when they did occur. These protozoan parasites can cause periodic outbreaks, unlike the soil-transmitted helminths which remain endemic at a fairly consistent prevalence rate within populations. Though *Giardia* has only been found at one site so far, *Entamoeba* was more common. *Entamoeba histolytica* causes severe diarrhoea or dysentery. In poor sanitary and crowded conditions it can cause outbreaks especially if drinking water becomes contaminated. Amoebic dysentery has a much higher mortality than other intestinal parasitic infections, and today it is estimated to be the third leading cause of death amongst parasitic diseases (Fletcher et al., 2012). People can become asymptomatic carriers of *Entamoeba* while still passing infective cysts. Aside from dysentery it can also cause ulcers in the colon and abscesses in other tissues such as the liver, brain, or lungs. Thus, it is expected that a small proportion of mortality from gastrointestinal disease in the Roman period was

from *Entamoeba histolytica*, especially in northern Europe where it has been found more commonly.

Other helminths found at multiple sites in the Roman period such as *Taenia* tapeworms and fish tapeworm also have varying symptoms depending on nutritional status of the host and intensity of infection. Fish tapeworm is more often linked to anaemia compared to other intestinal helminths, aside from hookworm, as it can cause B_{12} deficiency leading to fatigue and neurological symptoms such as peripheral neuropathy. It is estimated that one in five infections result in diarrhoea, abdominal pain, or more severe intestinal symptoms (Scholz et al., 2009). As in the case of roundworm and whipworm, these infections do not often cause death but can result in an individual's inability to work and decrease nutritional status potentially making them more susceptible to other diseases. This is similar for beef and pork tapeworm (*Taenia* sp.) which can lead to nutritional deficiencies in more severe cases and general abdominal upset. Though we do not know which species of *Taenia* were present in most cases, based on dietary preferences in the Roman period it is likely that both beef and pork tapeworm were present. *Taenia solium* (pork tapeworm) can result in another disease process, cysticercosis, if the human is used as the intermediate host for the worm. Neurocysticercosis is caused by the ingestion of *T. solium* eggs rather than larvae in pork, and then the larval form of the worm develops in cysts in neurological tissues such as the brain. This is often a chronic disease with symptoms showing up years after infection. As the cysts grow symptoms are often more severe and numerous neurological symptoms can occur (Garcia et al., 2005). Common clinical manifestations are seizures and cranial hypertension, which can result in focal neurological signs such as paralysis, vision changes, memory impairments, and poor coordination, all dependent on the area of the brain affected.

Neurocysticercosis is a common cause for of epilepsy in endemic countries today. It has been estimated that in endemic countries, about one third of patients with epilepsy also have neurocysticercosis (Gripper and Welburn, 2017). Furthermore, seizures occur in about 80% of individuals with neurocysticercosis (Carabin et al., 2011). There is evidence for cases of epilepsy in the Roman period, based on descriptions in historical texts (Magiorkinis et al., 2010). Two physicians, Galen and Soranus of Ephesus, described epilepsy symptoms and Galen notes that it is a disease of the brain (Magiorkinis et al., 2010). Though it is unclear how common the condition was, it is likely that some of these cases were a result of neurocysticercosis, especially in regions where *Taenia* sp. eggs have been recovered.

In general, morbidity from intestinal parasite infections is reliant on the number of worms an individual harbours in their intestines rather than simply the presence or absence of

worms. In modern populations this is usually estimated using eggs per gram in faecal samples. This type of quantification is challenging in past populations as we are not always dealing with simply faeces from one individual, often we have faecal samples from multiple individuals mixed together and mixed with other material. Furthermore, in modern populations it has been noted that there is strong heterogeneity in worm burdens, caused by yet unknown factors. What this means is that a small proportion of the population is more frequently and/or heavily infected (Hotez et al., 2008). This is a potential problem for palaeoparasitological studies as it makes it very difficult to link egg concentrations from certain types of samples, especially drains and latrines, to the level of infection in a community. When parasite eggs within a sample from the fill of a latrine or drain are quantified, we cannot determine if the eggs present represent the infection of a few heavily infected people or many people with minor infections. We do not know how many individuals would have used the latrine or the quantity of other types of waste and later soil added to the latrines, which all affect the concentration of eggs found. Therefore, pelvic soil samples are a better sample type for understanding how many people may have been infected with intestinal parasites. That being said, pelvic soil collected from skeletons can only give us information about the individuals who died with parasitic infection. Thus, as with the issues of studying diseases using skeletal remains we have a very skewed sample not necessarily representative of the living population. Furthermore, interpreting negative samples is still difficult as it is not always clear if parasite eggs simply did not preserve or if they were diluted out or simply not present in the small subsample studied. As is evident by the pelvic soil samples studied here, many more pelvic soil samples need to be studied in order to find evidence for infection compared to latrine and soil samples which may contain parasite eggs from multiple individuals. An ideal situation is having samples from latrines or drains within a settlement and also having pelvic soil samples from the same community.

In summary, quantifying the impact that these parasites may have had on the population is immensely difficult due to variations in clinical manifestations determined by host immunity, prior nutritional status, and intensity of infections. What can be said is that intestinal parasite infections would have had an impact on a proportion of the population, especially children, resulting in and exacerbating nutritional deficiencies, causing intellectual impairments, intestinal upset, and anaemia in fewer but more severe cases. While these symptoms and impacts of disease are not on the level of high mortality caused by plagues or malaria they would have been a constant presence in the lives of people living in the Roman Empire.

7 Limitations

It is important to note a number of limitations with the data presented and discussed here. Some have been mentioned throughout but will be elaborated on further now, and some pertain to the entirety of the research presented here. The limitations of this work will be discussed in a number of categories: 1) limitations as a result of the types of samples used; 2) limitations as a result of sample sizes and uneven geographic coverage; 3) limitations due to the dynamics of parasite preservation; and 4) limitations as a result of the sensitivity of methods used.

7.1 Sample Types

For this research the samples studied to find evidence for intestinal parasitic infection in the Roman period were varied, and similarly the samples that have been studied in previous work are equally varied. I analysed soil from latrines, drains, the pelvis of skeletons, and coprolites. Each of these sample types has unique characteristics that need to be considered when interpreting the presence of parasite eggs found within them.

First, soil from cesspits, latrines, or sewers connected to latrines give us a picture of parasites that may have infected anyone using those latrines or dumping waste into them. This is beneficial as we can study few samples to get a wider view of parasite infections within a community. However, it also makes it impossible to tie infection to any particular individual(s).

Certain considerations that need to be made for the Roman period is that some drains were still functional after abandonment of the site or use of latrines in the area. In many cases this may be to carry rain runoff. This is a potential problem as it means that any faecal material present in that drain from the Roman period could be lost over time or sediment moved in from other areas and time periods. While we have done our best to collect only undisturbed samples, sometimes it is unclear how long a drain may have been channelling water. Furthermore, it was a common practice to dump other waste into latrines or cesspits making it possible to recover parasites from the offal of animals. Cesspits and drains were not always covered or closed meaning that small rodents could get into them and we may also recover a small amount of rodent faeces which could contain parasite eggs. Thus while finding large numbers of eggs in cesspits or latrines primarily for human use most likely indicates human infection, we have to acknowledge that some eggs may have come from animals in the area. This is more of an issue when looking at generalist parasites that can infect many animals beside humans, such as *Capillaria* sp. and *Dicrocoelium* sp.

Pelvic soil samples are very valuable because when they do contain preserved eggs they can give us evidence for infection in a specific individual that we may have other demographic and pathological data about. This allows us to make links between different types of diseases present in a community and begin to look at frequency of infection in different age groups and sexes. However, yields tend to be lower when working with pelvic soil in comparison to latrine samples, though positive results have been found in a number of cases (Mitchell et al., 2013; Anastasiou et al., 2014; Yeh et al., 2016; Anastasiou et al., 2018). Furthermore, with pelvic soil we only have the potential of finding eggs from one individual and at one particular time, the time of death, whereas in latrines and sewers we can find eggs deposited from the faeces of multiple people which accumulate over many months or years of use.

Due to the fact that the individuals sampled are those that died, our samples are not reflective of the living population at the time of site occupation. The most commonly infected age group with soil-transmitted helminths in modern populations is school-age children (5–15 years) (Hotez and Kamath, 2009). In ancient populations with high infant mortality we may find a higher proportion of burials of infants. Infants who can make it past the age of five often have a higher life expectancy and may be found in cemeteries as adults. Thus, due to the fact that pelvic soil samples come from those who died, this sample type may result in us missing a number of infected individuals. This would be the case if previously infected individuals survived high risk ages and died later in adulthood, without an active infection. We will not be able to detect infections that these adults may have had earlier in life when they were in these higher risk groups.

Another consideration to be made when analysing pelvic soil samples is that the eggs recovered would have been present in the intestines of the individual, not yet excreted into the environment. This means that there was likely a combination of eggs in the process of being formed by the female worm as well as eggs already mixed with faeces ready to be excreted in the stool. Morrow and colleagues (2014) noted that a number of poorly preserved eggs in the intestines of mummies may have been a result of the fact that the eggs had not yet fully formed and thus were less resistant to degradation than eggs ready to be excreted or already excreted into the environment, as would be found in latrine and sewer samples. The various potential stages of development that eggs are found in in the intestines may make recovery of eggs lower in pelvic soil samples, especially if the bowels were not full at the time of death.

Compounded with this problem of poor recovery of eggs from pelvic soil samples is the distribution of infections within a population. As has been mentioned previously (see

section 5.1.2), helminths are often overdispersed in a population, meaning that a small proportion of the population carries the majority of the worms. This means that a larger proportion of the population would have low worm burdens or low levels of infection. Poor preservation and restrictions on the amount of soil that can be feasibly studied may result in us missing a number of these low level infections and only detecting infection in individuals who had the highest worm burdens. Utilizing additional methods to recover evidence for parasites may improve our chances of finding individuals with low levels of infection. For example, ancient DNA analysis can be used to detect DNA from parasites in sediment even when the entire egg is not preserved (Søe et al., 2018). However, only a few studies have used multiple methods and a good comparison of the rates of recovery of parasites using the different methods does not yet exist. In some cases microscopy can detect parasites that are not found using ancient DNA, and in others ancient DNA detects parasites that were not found using microscopy (Côté and Le Bailly, 2018). As ancient DNA methods become cheaper a widespread combination of microscopy, ELISA, and ancient DNA analysis may overcome some of these limitations.

Egg concentrations have been reported for all samples studied here, however, they should not be taken as an indication of the intensities or prevalence of infection at these sites. Unlike in modern studies where direct faecal samples are analysed, in the archaeological setting, aside from coprolites, our samples consist of faecal material mixed with an unknown amount of other organic material and sediment. This can vary widely between latrines, drains, and even in pelvic soil samples and is dependent on a variety of factors including addition of other materials to these areas in antiquity, loss of eggs over time due to degradation, erosion, percolation of eggs in soil, and variations in concentrations in eggs between subsamples studied from the same sample. Furthermore, when dealing with samples from cesspits, latrines, or drains, the eggs found represent eggs in the faeces of multiple individuals over a long period of time who may have had varying intensities of infection both between individuals and over time.

While incorporating a variety of samples means we need to be careful about the interpretation of the eggs present, this variety of samples also has benefits. One is that by varying the types of samples used we can get both an indication of disease throughout individual's lives and wider time periods, as would come from latrines, drains and coprolites, whereas from pelvic soil we can get an indication of disease at the time of death. Furthermore, analysis of pelvic soil samples from cemeteries can give us an idea of infection in the individuals who lived at a certain place (for an unknown period of time), while with samples

from latrines and sewers we can also pick up infections in people who were moving around and possibly only present in the area for a very short period of time, long enough to deposit their stool in a latrine. In this way, multiple sample types from the same site become very valuable in understanding the intricacies of infections in a wider community.

7.2 Sample Sizes and Uneven Geographic Coverage

One of the main goals of this research was to determine if there were variations in parasitic infection in different regions of the Roman Empire. Prior to the start of this work there were some areas of the empire where very few previous studies or no studies had been done. Thus this work provided some of the first evidence for parasitic infection in the Balkans in the Roman period and added to the one or two sites that had been studied in Italy and Turkey. This has allowed for an initial discussion of the dynamics of parasitic infection in the Eastern Mediterranean and Italy and a comparison to regions that have seen more work, such as Britain and France. One pattern that became evident was the predominance of faecal-oral parasites in Italy and the eastern Mediterranean and the absence of other zoonotic parasites such as tapeworms and liver flukes that are found in the frontier provinces and Britain.

A consideration that needs to be taken with this is that the data are not distributed equally across the Roman Empire. Even with the additional samples studied here, more samples have been studied from Britain and France compared to the Mediterranean region. This means that these patterns are subject to change if more samples are studied from these regions. It is possible that so far I have only found the most common parasites in these regions and that as more samples are studied some of the less common parasites will be picked up.

In addition to this, the variations in latrine structures, population sizes, and building materials means that the sample types studied in the different regions are slightly different. In Britain many parasites studies have been done on soil from pits and wells. This is uncommon in the Mediterranean region where latrine structures are preserved in major Roman cities and thus samples can be taken from latrines and tile-lined drains that still have their original building materials present. It is possible that parasites preserve differently in simple soil pits compared to lined latrines or cesspits and drains.

7.3 Dynamics of Parasite Preservation

Many samples studied did not have any parasite eggs present them, namely the pelvic soil from Çatalhöyük, Boncuklu, Winterborne, Lauriacum, and Vagnari. It is possible that none of these individuals were infected at the time of death. It also possible that parasite eggs in these areas simply did not preserve. Furthermore, in the samples that did contain some parasite eggs

it is possible that eggs from other species did not survive the past 2,000 years and thus were not represented in the samples.

Relatively little is understood about factors that can affect parasite preservation compared to skeletal material and biomolecules like DNA and proteins. For skeletal material it is known that constant exposure to water, and fluctuating levels of water negatively impacts preservation. Additionally, acidic conditions result in chemical changes and destruction of the bone matrix (Brown and Brown, 2011:94). Typically desiccated materials are very well preserved, that is similar for bones, tissues, and plant remains. Waterlogged conditions result in good preservation of plant remains, wood, and other artefacts likely due to the anaerobic conditions of waterlogged environments which prevent oxidative decay. This seems to be true for parasite eggs as well, where waterlogged conditions have resulted in exceptional preservation of a range of taxa from sites such as Must Farm (see section 4.2.1) as well as at other lakeside settlements (Maicher et al., 2017; Maicher et al., 2019). It was notable that nearly all *Echinostoma* eggs in the Must Farm coprolites were folded and broken and none were present in the soil samples studied. This indicates that they may not preserve well except in very specific conditions achieved in the waterlogged coprolites, and these conditions were not even met in sediment at the site. More sites in northern Europe have waterlogged conditions which may be partially responsible for the higher species diversity found in this region.

The impact of soil acidity in the diagenesis of parasite eggs has not been widely studied, though we might expect that eggs could survive a large range of acidity as they need to pass through the gastrointestinal tract which has pH ranging from 2 in the stomach to 7 in the small and large intestines. Most intestinal parasite eggs hatch in the small intestines and it has been shown that exposing eggs to a pH between 6 and 8.3 can induce hatching (Rogers, 1960). However, recent studies have also shown that certain gut microbiota, such as *E. coli,* provide important cues for inducing egg hatching (Vejzagić et al., 2015a). Survival of these parasite species relies on hatching in the correct area of the gastrointestinal tract and thus it is not expected that hatching in the environment is common. Certain parasite eggs such as those from hookworm (*Ancylostoma* sp. and *Necator americanus*) hatch in the soil where their larvae then develop and can infect humans via passage through the skin. This is one possible reason why hookworm eggs are not commonly found in archaeological samples, as the larvae do not preserve and unless development is arrested before the eggs are fully embryonated the eggs would not be found in the soil (Bouchet et al., 2003).

The structure of parasite egg shells generally consists of multiple layers including an outer vitelline layer made up of lipoproteins, then a chitinous layer with varying density and orientation of protein fibrils, and an inner lipid layer (Wharton, 1980). Eggs of some genera such as *Capillaria* sp. and *Trichuris* sp. also have polar plugs on opposing ends. The structure of the polar plugs is similar to the rest of the egg shell but the protein level in the chitinous layer is lower (Vejzagić et al., 2015b). The chitinous layer is what gives the egg shell strength and structure and is expected to be important for preservation of the eggs in archaeological samples. Chitin is a polymer found in many organisms such as the exoskeletons of arthropods and the walls of fungal spores (Rinaudo, 2006). The molecular organization of the chitinous layer of the egg shell varies in different genera of parasites, mostly in its protein content and orientation, as well as thickness (Wharton, 1980). Chitin is broken down in the environment by chitinase producing bacteria and fungi. These include many bacteria found in soil including genera such as *Bacillus*, *Pseudomonas, Streptomyces,* and *Vibrio* (Makarios-Laham and Lee, 1995; Beier and Bertilsson, 2013), suggesting that soil bacteria likely play a role in degradation of parasite eggs. This means that the composition of bacteria in various soil types, which can also depend on temperature and pH, may affect preservation of eggs in different environments. Analysis of fossil arthropods has shown that mineralization and preservation of chitinous tissues may actually be a result of replacement of chitin with more resistant molecules from the surrounding sediment (Gupta et al., 2006), also highlighting the role of sediment composition in potential preservation of eggs. Mineralization rates of chitin appear to be higher in freshwater ecosystems (Beier and Bertilsson, 2013), which may in part explain the high taxonomic diversity of parasites recovered from lakeside settlements in Europe.

Other factors that may contribute to the loss of parasite eggs over time are predation by fungi, mites, and other insects (Morrow et al., 2016). Eggs have been shown to percolate through soil over time to be displaced from their original deposition, however this process is quicker and most problematic in particularly rainy and sandy environments (Camacho et al., 2016), and should be less of an issue in closed contexts such as lined latrines and drains.

In reference to the results presented here, this means that there may be a number of species that were not detected in the samples studied but that were present in these Roman communities due to egg structure or the life cycle of the parasites. For example, some eggs do not typically preserve very well such as those from hookworm, because it hatches in the soil in order for larvae to penetrate the skin. For other taxa such as *Fasciola* and *Echinostoma* their life cycles may in part be responsible for them not being found at certain sites. These parasite eggs need to hatch so that larvae can find their proper intermediate hosts in aquatic

environments, thus we may only find hatched eggs or fewer eggs in general. On the other hand, eggs from pinworm (*Enterobius vermicularis*) may have not been preserved because they have thin eggshells. Based on the widespread presence of other soil-transmitted helminths, roundworm and whipworm, we might reasonably expect that hookworm was also present in some of the study sites that are in warm moist climates where the parasite can survive, such as in the Mediterranean. Similarly, pinworm is one of the most common helminths to infect children today and it is easily passed between people through unwashed hands and likely would have spread easily in Roman communities. However, taxa such as *Taenia* sp. that have very thick egg shells would be expected to preserve as well as *Ascaris* and *Trichuris*. This means that in areas where *Ascaris* and *Trichuris* were found, I would expect *Taenia* could be recovered if it was present in the samples.

Due to indications that soil type (pH, bacterial and fungal content, presence of other organic material), temperature, and water content may impact chitin degradation and mineralization it should be noted that the higher diversity of parasites found in northern Europe may in part be due to waterlogged samples in these regions and lower temperatures. However, the dynamics of parasite egg preservation in these different regions is not well understood and needs to be explored further in order to separate this from the role of differences in anthropic factors and ecology that may result in variations in species causing infections in these regions.

7.4 Sensitivity of Methods in Palaeoparasitology

Determining the precise sensitivity of methods used in archaeological research is not possible as we do not have known samples that can be used to determine the sensitivity of methods. Rather in most cases sensitivities are determined in modern tests or estimated using experimental studies and results need to be reported with these limitations in mind. This is the same for microscopy and ELISA used in palaeoparasitology. While the sensitivity and specificity of the ELISA kits are known (see section 3.3), these values are reliant on the tests being used for their intended purpose (on modern faecal samples). When we apply the same tests to archaeological sediments we have to expect that the sensitivity will be much lower. There are likely numerous samples that have antigens present in them but do not test positive for a number of possible reasons. These reasons include, changes to the structure of proteins resulting in their inability to bind to the antibodies in the kits, low concentrations of antigens which are not able to trigger a positive result, and other compounds in the soil interacting with the reagents in the kits. While false positives can also be an issue, resulting in decreased

specificity, these are expected to be much lower due to the use of highly specific antibodies that should only bind to the antigen they are targeting.

In the case of microscopy, sensitivity and specificity of the methods are largely unknown. There are certainly numerous samples that at one point had parasite eggs in them but these did not survive to be found or the eggs have been broken/damaged to the point where they are not recognized by the observer. As mentioned above, the infection dynamics of intestinal helminths also influences our ability to recover them in the archaeological record. This is particularly the case for studies using pelvic soil samples. Due to the fact that helminth infections are often overdispersed within a population (typically it is estimated that 20% of a population harbours 80% of the worm population [Hotez et al., 2006]), we would expect a large proportion of the population to have low worm burdens or no infection, while only a small proportion has high worm burdens. This in combination with the loss of parasite eggs over time likely results in us missing a number of infections in individuals who have these low worm burdens.

One way to increase our chances of recovering evidence for all helminths and protozoa that were present in a sample is to augment our studies with additional methods. One such method that is being used in some initial studies is ancient DNA. In recent years, ancient DNA studies have adapted methods for analysis of environmental DNA used by ecologists. These methods allow for a broad characterization of plants, animals, microorganisms, and eukaryotic parasites present in archaeological samples (Andersen-Carpenter et al., 2011; Willerslev et al., 2014; Pedersen et al., 2015). Using targeted approaches (e.g. PCR metabarcoding or hybridization capture) a specific set of organisms can be focused on to decrease costs of these types of analysis, making them more accessible to palaeoparasitological research. There is evidence that in cases where eggs are not identified under the microscope, DNA from parasites can still be recovered from archaeological samples (Søe et al., 2018; Côté and Le Bailly, 2018). However, at the same time there are also cases where eggs are identified under the microscope but DNA from those same parasites is not recovered (Søe et al., 2018). This exemplifies the benefits of combining methods in order to increase our chances of recovering the full taxonomic profile of a sample.

8 Future Directions

With the completion of this research a number of avenues for future research have been highlighted or remain important for future research in palaeoparasitology in general, and in investigating parasitic infection in the Roman period.

First, although this research aimed to increased comparative data for parasites in Bronze Age and Iron Age Europe there is still a long way to go before these time periods have been as extensively studied as the Neolithic period and later Roman and Medieval periods. Especially in the Mediterranean region there is almost no information about parasitic infection in Bronze Age and Iron Age populations. This information is extremely valuable for tracing the changes in parasite infection through time in the region, and putting the Roman period material into context.

In addition, focusing on transition periods can be valuable in understanding changes in parasitic infection through time. In particular having samples from different time periods at the same site can allow for a better understanding of how changing cultural practices and social structures may be impacting parasite transmission in ancient communities.

As has been shown by this research, in some cases large numbers of individuals need to be studied in order to find positive results from pelvic soil samples. Thus, continual collection and analysis of samples will be needed in order to have enough data to look for patterns of infection at the individual level. This continual study of pelvic soil samples will hopefully allow us to get to the point where we can integrate information about parasite infections with demographic information and other pathologies recorded from skeletal remains. This will allow us to answer questions such as: what infections commonly co-occur with intestinal parasite infections?; are there differences in frequency of infection in males and females, or individuals of different ages?; how might burial treatment impact recovery of parasite eggs?; do certain parasites commonly co-occur in the same individual?; how often did polyparasitism occur in the past?

Finally, in order to circumvent some of the limitations with common methods including microscopy and ELISA, used in palaeoparasitology—and in this research, using additional methods, namely ancient DNA will likely be an advantageous addition. One major limitation of the methods used here was that in many cases parasite eggs could only be taxonomically identified to the genus level, rather than the species level. Furthermore, there are a number of eggs which have been shown to preserve poorly and thus it seems likely that current methods are not detecting the full range of parasites causing disease in past populations. Genetic analysis of archaeological samples containing intestinal parasites

provides us with the ability to accurately identify parasites to the species level and identify additional parasites present in sediment whose eggs did not preserve to be visualized. In addition, aDNA analysis can allow us to reconstruct genomes of ancient parasites that can be used to trace the evolution of these parasites alongside human beings. Initial studies analysing human parasites using aDNA have shown promise though parasites are still a novel pathogen to study using aDNA (Côté and Le Bailly, 2018; Søe et al., 2018). One of the main reasons for this has been the lack of modern genetic sequences from parasites of interest which are necessary references to be able to identify ancient sequences. Ancient DNA analysis of samples studied here is already underway as a continuation of this project. Recent optimization of methods for sedimentary DNA extraction, largely used in environmental DNA studies, and targeted enrichment are showing some very early promising results for characterization of parasites in some of the samples studied as part of this research. These methods can allow for taxonomic identification to the species level in cases where microscopy only allowed for genus level identifications. In some cases, ancient DNA can also recover DNA from parasites in cases where eggs were not seen under the microscope, possibly because they were broken or not preserved.

9 Conclusions

This doctoral research has provided the first evidence for parasitic infection in unstudied regions of the Roman Empire including Serbia and Cyprus. It has also contributed evidence to regions where very few studies had been done previously including Turkey and Italy. Furthermore it has contributed to our understanding of parasitic infection in prehistoric communities, namely people living in the Late Bronze Age marshland of Britain, as well as the first evidence for parasite infection in people living in Italy in the Lombard period. Analysing samples from 12 Roman period sites, it has provided evidence for parasites from nine sites in addition to the 53 that have already provided preserved parasite remains. It has further ventured to discuss these data in relation to archaeological and historical evidence that provides the context necessary to understand environmental, cultural, and social factors involved in the establishment and maintenance of these pathogens in Roman communities.

The conclusions from this research will be presented by revisiting the research questions that were described in section 1.2. The results obtained allow us to provide answers to most questions that guided this research though some can be answered more thoroughly than others.

Starting with the broadest question; what taxa of parasites were present in the Roman Empire? A total of 11 taxa of parasites have been found in Roman period sites including *Capillaria* sp., *Dicrocoelium* liver fluke, *Echinococcus* sp., *Entamoeba histolytica*, *Fasciola* liver fluke, fish tapeworm, *Giardia duodenalis*, *Macracanthorhynchus* sp., pinworm, roundworm, *Taenia* tapeworms, *Toxocara* sp., and whipworm. These come from the newly studied sites and previously studied sites. Roundworm and whipworm are the most common parasites found across the Roman Empire. In fact, they are the dominant taxa found in regions focused on here including Italy and Turkey. In these two areas there is no evidence for any other species except for gastrointestinal protozoa and *Fasciola* found in a sediment core. This highlights a pattern that starts to become evident with the newly studied sites in the Mediterranean region. In these areas, predominantly and almost exclusively faecal-oral parasites that rely on poor sanitation and hygiene have been found. This is in conjunction with an absence of taxa that can be considered zoonotic, either having animals as a necessary intermediate host or that are commonly transmitted between humans and animals, such as *Fasciola* sp., *Dicrocoelium* sp., *Taenia* sp., and *Diphyllobothrium* sp. which have been found at sites north of the Mediterranean.

Numerous ecosocial factors that may explain this variation in parasite presence in different areas of the empire have been explored. The predominance of soil-transmitted

helminths, namely roundworm and whipworm, across the empire is likely tied to design and use of sanitation infrastructure and methods for collecting, disposing, and even reusing human excrement. Some of these may have been larger issues in heavily urbanized areas of Turkey and Italy, where disposal of human waste may have been harder in densely populated cities. Variations in diet and human-animal interactions have been explored to consider differences in the presence of zoonotic parasites across the empire. Preferences for certain types of meat and fish, as well as cooking styles likely contributed to differences in the transmission of tapeworms. Furthermore, wider site ecology likely has a strong influence on taxa present. Though it is difficult to determine the degree to which each of these numerous factors may have played a role in parasite transmission, they highlight ample routes that existed for the spread of parasitic diseases in Roman communities. Finally, the role of differential preservation of parasite eggs has been discussed and may also be contributing to some of the patterns seen so far.

This research has also highlighted some of the difficulties in working with pelvic soil to track parasite infections in the past. Mainly, that many more samples need to be analysed to find evidence for infection, and that yields are much lower compared to working with the fill of latrines or drains. It is for this reason that there are still not enough data to explore variations in infection in age groups or between the sexes.

Comparing the evidence gathered for parasites in the Roman Empire to what is known about parasites prior to and after the fall of the Roman Empire has highlighted some trends in parasitic infection across these longer timescales. In general, there is a trend of decreasing taxonomic diversity from the Neolithic period to the Roman period, with more zoonotic parasites found in Neolithic, Bronze Age, and Iron Age sites. In the Roman period we start to see the predominance of parasites linked to poor sanitation and hygiene such as soil-transmitted helminths with a decrease in the proportion of sites where eggs of liver flukes and tapeworms were found. The taxonomic diversity seen in the Roman period is similar to that in the Medieval period. Increased population sizes, urbanization, and a diet reliant on farmed animals likely has a strong influence on these patterns. It may be, that in prehistoric periods the presence of rare zoonotic taxa may reflect wild animals making up a larger portion of the diet; whereas strong ideas about thorough cooking of meats and consumption of heavily salted meat and fish may have decreased infections with tapeworms in parts of the Roman Empire. Fewer people interacting closely with animals in heavily urbanized centres may have further reduced infections with taxa such as liver flukes in the Roman period.

Intestinal parasites, especially helminths, almost certainly did not go unnoticed. Parasites are described by multiple writers at the time including Galen and Celsus. Furthermore, numerous remedies were suggested for their treatment by these physicians as well as other authors such as Pliny the Elder and Cato the Elder, some of which may have even been effective at high enough doses. The data collected from the new studied sites and previous publications show that parasite infections were a fairly common occurrence in Roman communities in the past and would have contributed to the burden of disease across the empire.

10 Publications

Below is a list of publications from the research presented here as well as publications from other projects I completed or contributed to during my time in the Ancient Parasites Laboratory at the University of Cambridge as a PhD student. These are presented in chronological order with the most recent first.

10.1 Published and Accepted Articles

Ledger, M.L. & Mitchell, P.D. Evolutionary perspectives on human parasitic infection, from ancient parasites to modern medicine. In: Plomp, K., Roberts, S., Elton, S. & Bentley, G. (eds.) *Evolving health: Paleopathology and evolutionary medicine.* Oxford, Oxford University Press, (accepted).

Ledger, M.L., Grimshaw, E., Fairey, M. & Mitchell, P.D. Parasitological analysis of sediments and coprolites from the Must Farm settlement. In: Ballantyne, R. & Knight, M. (eds.) *Excavations at Must Farm*. London, English Heritage, (accepted).

Ledger, M.L. & Mitchell, P.D. (2020) Review of: Koloski-Ostrow, A.O. The Archaeology of Sanitation in Roman Italy: Toilets, Sewers, and Water Systems. University of North Carolina Press 2015. *Isis.* 111(1), 156–158.

Eskew, W.H., Lloyd, L.C., Pyles, G.C., **Ledger, M.L.**, Gosker, J. & Mitchell, P.D. (2020) Intestinal parasites from an Ottoman period latrine in Acre (Israel) dating to the early 1800s CE. *Korean Journal of Parasitology.* 57(6), 575–580.

Knorr, D., Smith, W., **Ledger, M.L.**, Peña-Chocarro, L., Pérez-Jordà, G., Clapés, R., Palma, M. & Mitchell, P.D. (2019) Intestinal parasites in six Islamic medieval period latrines from 10th–11th century Córdoba (Spain) and 12th–13th century Mértola (Portugal). *International Journal of Paleopathology*. 26, 75–83.

Ledger, M.L., Anastasiou, E., Shillito, L.-M., Mackay, H., Bull, I.D., Haddow, S.D., Knüsel, C.J. & Mitchell, P.D. (2019) Parasite infection at the early farming community of Çatalhöyük. *Antiquity*. 93 (369), 573–587.

Ledger, M.L., Grimshaw, E., Fairey, M., Whelton, H.L., Bull, I.D., Ballantyne, R., Knight, M. & Mitchell, P.D. (2019) Intestinal parasites at the Late Bronze Age settlement of Must Farm, in the fens of East Anglia, UK (9th century B.C.E.). *Parasitology*. 146 (12), 1583–1594.

Ledger, M.L. & Mitchell, P.D. (2019) Tracing zoonotic parasite infections throughout human evolution. *International Journal of Osteoarchaeology*. 1–12, doi:10.1002/oa.2786.

Ledger, M.L. & Mitchell, P.D. (2019) Review of: Drancourt, M., Raoult, D. (eds) Paleomicrobiology of Humans. American Society for Microbiology Press, 2016. *American Journal of Human Biology.* 31, e23202.

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). *Journal of Archaeological Science: Reports*. 21, 289–297.

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

10.2 Articles Under Review and In Preparation

Ledger, M.L., Rowan, E., Gallart Marques, F., Sigmier, J.H., Šarkić, N., Redžić, S., Cahill, N.D. & Mitchell, P.D. Intestinal parasitic infection in the eastern Mediterranean during the Roman period. *American Journal of Archaeology*. (under review).

Murchie, T.J., Kuch, M., Duggan, A., **Ledger, M.L.**, Roche, K., Klunk, J., Karpinski, E., Hackenberger, D., Sadoway, T., MacPhee, R., Froese, D. & Poinar, H. (2019) PalaeoChip Arctic1.0: An optimised eDNA targeted enrichment approach to reconstructing past environments [preprint]. *BioRxiv*. Available at: doi.org/10.1101/730440. (under review in Quaternary Science).

Deforce, K., Derreumaux, M., Goffette, Q., Henrotay, D., **Ledger, M.L.**, Mitchell, P.D. & Pigière, F. Diet, hygiene and health in Roman age northern Gaul. A multidisciplinary study of a latrine from Arlon (c. 250–280 AD, S-Belgium). *Journal of Archaeological Science: Reports.* (in prep).

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Al-Mathal, E.M. & Alsalem, A.M. (2012) Pomegranate (*Punica granatum*) peel is effective in a murine model of experimental *Cryptosporidium parvum*. *Experimental Parasitology.* 131(3), 350–357.

Anastasiou, E. (2015) Parasites in European populations from prehistory to the Industrial Revolution. In: Mitchell, P.D. (ed.). *Sanitation, latrines and intestinal parasites in past populations*. Farnham, Ashgate. pp. 203–217.

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Appendix A: Publications

A.1 Must Farm (Parasitology)

Ledger, M.L., Grimshaw, E., Fairey, M., Whelton, H.L., et al. (2019) Intestinal parasites at the Late Bronze Age settlement of Must Farm, in the fens of East Anglia, UK (9th century B.C.E.). *Parasitology*. 146 (12), 1583–1594.

A.2 Çatalhöyük (Antiquity)

Ledger, M.L., Anastasiou, E., Shillito, L.-M., Mackay, H., et al. (2019) Parasite infection at the early farming community of Çatalhöyük. *Antiquity*. 93 (369), 573–587.

A.3 Tracing Zoonotic Parasites Throughout Human Evolution (International Journal of Osteoarchaeology)

Ledger, M.L. & Mitchell, P.D. (2019) Tracing zoonotic parasite infections throughout human evolution. *International Journal of Osteoarchaeology*. doi:10.1002/oa.2786.

A.4 Ephesus (Journal of Archaeological Science: Reports)

Ledger, M.L., Stock, F., Schwaiger, H., Knipping, M., et al. (2018) Intestinal parasites from public and private latrines and the harbour canal in Roman Period Ephesus, Turkey (1st c. BCE to 6th c. CE). *Journal of Archaeological Science: Reports*. 21, 289–297.

A.5 Parasites in the Eastern Mediterranean (American Journal of Archaeology)

Ledger, M.L., Rowan, E., Gallart Marques, F., Sigmier, J.H., Šarkić, N., Redžić, S., Cahill, N.D., Mitchell, P.D. (under review) Intestinal parasitic infection in the Eastern Mediterranean during the Roman period. *American Journal of Archaeology*.

Appendix B: Raw Data and Statistical Calculations

B.1 ELISA Absorbance Values from Must Farm

Table B1.1: Absorbance values from *Cryptosporidium* ELISA (test 1) for Must Farm sediment samples. Positive samples are highlighted in yellow. Positive and negative control are in red boxes.

	Upstream control	Inside str. 1 (occ.)	inside str. 2 (occ.)	inside str. 3 (occ.)	inside str. 4 (occ.)	outside str. 3 (occ.)	inside str. 1 (post-conf)	inside str. 3 (post-conf)	Downstream Control
	1	2	3	4	5	9	10	11	12
A	0.075	0.042	0.066	0.041	0.042	0.041	0.167	0.065	0.51
B	0.045	0.042	0.061	0.041	0.04	0.041	0.049	0.054	0.04
C	0.045	0.043	0.039	0.041	0.037	0.054	0.052	0.055	0.049
D	0.052	0.038	0.038	0.039	0.04	0.041	0.056	0.044	0.037
E	0.043	0.034	0.048	0.036	0.036	0.054	0.084	0.05	0.037
F	0.037	0.038	0.043	0.036	0.038	0.048	0.039	0.043	0.042
G	0.04	0.047	0.045	0.036	0.036	0.057	0.044	0.07	0.048
H	0.044	0.197	0.043	0.035	0.037	0.043	0.052	0.039	0.119

Table B1.2: Absorbance values from *Cryptosporidium* ELISA (test 2) for Must Farm sediment samples. Positive samples are highlighted in yellow. Positive and negative control are in red boxes.

	Upstream control	Inside str. 1 (occ.)	inside str. 2 (occ.)	inside str. 3 (occ.)	inside str. 4 (occ.)	outside str. 3 (occ.)	inside str. 1 (post-conf)	inside str. 3 (post-conf)	Downstream Control
	1	2	3	4	5	9	10	11	12
A	0.037	0.035	0.04	0.037	0.042	0.034	0.035	0.037	0.352
B	0.035	0.04	0.033	0.033	0.034	0.036	0.037	0.033	0.034
C	0.035	0.034	0.034	0.035	0.034	0.035	0.037	0.035	0.035
D	0.037	0.043	0.032	0.032	0.037	0.037	0.033	0.043	0.037
E	0.034	0.035	0.034	0.033	0.034	0.04	0.035	0.037	0.044
F	0.035	0.03	0.032	0.034	0.033	0.038	0.036	0.035	0.036
G	0.034	0.034	0.034	0.034	0.039	0.033	0.034	0.036	0.041
H	0.039	0.033	0.036	0.034	0.038	0.034	0.034	0.036	0.034

Table B1.3: Absorbance values from *Entamoeba* ELISA (test 1) for Must Farm sediment samples. Positive samples are highlighted in yellow. Positive and negative control are in red boxes.

	Upstream control	Inside str. 1 (occ.)	inside str. 2 (occ.)	inside str. 3 (occ.)	inside str. 4 (occ.)	outside str. 3 (occ.)	inside str. 1 (post-conf)	inside str. 3 (post-conf)	Downstream Control
	1	2	3	4	5	9	10	11	12
A	0.044	0.033	0.045	0.037	0.032	0.06	0.063	0.065	1.572
B	0.045	0.048	0.041	0.047	0.046	0.046	0.045	0.045	0.044
C	0.046	0.048	0.041	0.042	0.043	0.047	0.045	0.048	0.04
D	0.049	0.049	0.048	0.044	0.06	0.049	0.041	0.043	0.045
E	0.214	0.052	0.043	0.052	0.048	0.046	0.055	0.043	0.04
F	0.18	0.48	0.519	0.342	0.551	0.047	0.046	0.045	0.055
G	0.879	1.136	0.931	0.557	0.641	0.042	0.051	0.047	0.045
H	1.729	1.884	1.914	2.55	1.649	1.166	0.046	0.048	0.04

Table B1.4: Absorbance values from *Entamoeba* ELISA (test 2) for Must Farm sediment samples. Positive samples are highlighted in yellow. Positive and negative control are in red boxes.

	Upstream control	Inside str. 1 (occ.)	inside str. 2 (occ.)	inside str. 3 (occ.)	inside str. 4 (occ.)	outside str. 3 (occ.)	inside str. 1 (post-conf)	inside str. 3 (post-conf)	Downstream Control
	1	2	3	4	5	9	10	11	12
A	0.035	0.053	0.05	0.063	0.045	0.043	0.043	0.04	0.501
B	0.043	0.04	0.038	0.047	0.036	0.034	0.045	0.039	0.054
C	0.042	0.04	0.045	0.038	0.043	0.04	0.048	0.042	0.036
D	0.04	0.044	0.059	0.037	0.055	0.04	0.045	0.036	0.037
E	0.039	0.037	0.043	0.039	0.036	0.035	0.041	0.037	0.038
F	0.037	0.048	0.04	0.053	0.042	0.035	0.036	0.035	0.036
G	0.087	0.048	0.039	0.034	0.058	0.037	0.042	0.042	0.038
H	0.087	0.045	0.043	0.033	0.036	0.043	0.036	0.044	0.046

Table B1.5: Absorbance values from *Giardia* ELISA (test 1) for Must Farm sediment samples. Positive samples are highlighted in yellow. Positive and negative control are in red boxes.

	Upstream control	Inside str. 1 (occ.)	inside str. 2 (occ.)	inside str. 3 (occ.)	inside str. 4 (occ.)	outside str. 3 (occ.)	inside str. 1 (post-conf)	inside str. 3 (post-conf)	Downstream Control
	1	2	3	4	5	9	10	11	12
A	1.001	1.105	0.068	1.741	1.511	2.387	0.569	0.033	0.97
B	0.303	0.618	0.093	1.074	0.163	0.044	0.829	0.043	0.043
C	0.052	0.341	2.014	0.674	0.307	2.049	0.045	0.047	0.045
D	0.393	1.027	0.443	0.871	0.455	1.048	1.303	0.043	0.045
E	0.771	0.766	0.79	0.043	0.041	0.453	0.039	0.045	0.044
F	0.048	0.045	0.721	0.047	0.045	1.996	0.489	0.044	0.045
G	0.047	0.035	0.08	0.045	0.044	1.413	0.112	0.045	0.046
H	0.047	0.035	0.038	0.047	0.044	0.045	0.034	0.036	0.046

Table B1.6: Absorbance values from *Giardia* ELISA (test 2) for Must Farm sediment samples. Positive samples are highlighted in yellow. Positive and negative control are in red boxes.

	Upstream control	Inside str. 1 (occ.)	inside str. 2 (occ.)	inside str. 3 (occ.)	inside str. 4 (occ.)	outside str. 3 (occ.)	inside str. 1 (post-conf)	inside str. 3 (post-conf)	Downstream Control
	1	2	3	4	5	9	10	11	12
A	0.04	0.03	0.048	0.051	0.053	0.051	0.048	0.053	0.852
B	0.048	0.052	0.039	0.04	0.049	0.052	0.069	0.048	0.048
C	0.06	0.057	0.049	0.049	0.063	0.054	0.049	0.051	0.049
D	0.057	0.051	0.045	0.048	0.054	0.048	0.048	0.057	0.052
E	0.057	0.052	0.059	0.05	0.052	0.052	0.063	0.047	0.051
F	0.064	0.062	0.084	0.052	0.058	0.051	0.046	0.049	0.043
G	0.06	0.065	0.047	0.051	0.064	0.054	0.047	0.044	0.058
H	0.047	0.046	0.04	0.04	0.046	0.046	0.053	0.044	0.045

Table B1.7: Absorbance values from *Cryptosporidium* ELISA (test 1) for Must Farm coprolites. Positive samples are highlighted in yellow. Positive and negative control are in red boxes.

	Coprolite <3127>	Coprolite <3631>	Coprolite <3642>	Coprolite <3082>	Coprolite <4029>
	8	9	10	11	12
A	0.209	1.042	1.508	0.857	1.472
B	0.28	0.252	0.696	0.34	0.048
C	0.256	0.166	0.398	0.311	0.117
D	0.575	0.152	0.417	1.932	0.1
E	0.187	0.156	0.903	0.306	0.159
F	0.196	0.138	0.183	0.915	0.702
G	0.146	0.149	0.257	0.849	1.325
H	0.801	0.188	0.568	1.084	1.208

Table B1.8: Absorbance values from *Cryptosporidium* ELISA (test 2) for Must Farm coprolites. Positive samples are highlighted in yellow. Positive and negative control are in red boxes. Note some coprolites are different because there was not enough to run all twice.

	Coprolite <3080>	Coprolite <3177>	Coprolite <3642>	Coprolite <3492>	Coprolite <4029>
	8	9	10	11	12
A	0.169	0.134	0.11	0.135	1.645
B	0.198	0.171	0.113	0.164	0.051
C	0.175	0.226	0.109	0.153	0.096
D	0.202	0.178	0.185	0.156	0.091
E	0.193	0.165	0.111	0.141	0.158
F	0.252	0.17	0.104	0.132	0.15
G	0.299	0.157	0.105	0.168	0.169
H	0.185	0.188	0.09	0.127	0.178

Table B1.9: Absorbance values from *Entamoeba* ELISA (test 1) for Must Farm coprolites. Positive samples are highlighted in yellow. Positive and negative control are in red boxes.

	Coprolite <3127>	Coprolite <3631>	Coprolite <3642>	Coprolite <3082>	Coprolite <4029>
	8	9	10	11	12
A	0.761	0.348	1.757	1.295	1.731
B	1.453	0.449	2.412	1.647	0.042
C	1.022	0.321	2.278	1.458	0.707
D	1.252	0.418	2.154	1.097	0.914
E	1.145	0.714	2.382	1.14	0.708
F	0.56	0.379	2.426	1.066	0.588
G	0.786	0.407	1.942	0.9	0.332
H	1.144	0.587	2.646	1.641	1.364

Table B1.10: Absorbance values from *Entamoeba* ELISA (test 2) for Must Farm coprolites. Positive samples are highlighted in yellow. Positive and negative control are in red boxes. Note some coprolites are different because there was not enough to run all twice.

	Coprolite <3080>	Coprolite <3177>	Coprolite <3642>	Coprolite <3492>	Coprolite <4029>
	8	9	10	11	12
A	0.667	0.058	0.197	0.457	2.298
B	0.858	0.821	0.126	0.085	0.033
C	0.765	0.6	0.198	0.136	0.238
D	0.8	0.651	0.171	0.141	0.199
E	0.746	0.308	0.13	0.183	0.19
F	0.838	0.508	0.119	0.209	0.23
G	0.882	0.268	0.08	0.404	0.232
H	1.019	0.203	0.261	0.094	0.258

Table B1.11: Absorbance values from *Giardia* ELISA (test 1) for Must Farm coprolites. Positive samples are highlighted in yellow. Positive and negative control are in red boxes.

	Coprolite <3127>	Coprolite <3631>	Coprolite <3642>	Coprolite <3082>	Coprolite <4029>
	8	9	10	11	12
A	0.378	0.431	0.495	0.977	2.528
B	1.072	0.359	0.54	0.998	0.053
C	0.352	0.383	0.937	0.723	0.68
D	0.341	0.346	0.415	0.722	0.267
E	0.394	0.419	1.169	0.779	0.24
F	0.269	0.392	0.467	0.817	0.206
G	0.965	0.47	0.593	0.742	0.188
H	0.829	0.427	1.199	0.78	0.29

Table B1.12: Absorbance values from *Giardia* ELISA (test 2) for Must Farm coprolites. Positive samples are highlighted in yellow. Positive and negative control are in red boxes. Note some coprolites are different because there was not enough to run all twice.

	Coprolite <3080>	Coprolite <3177>	Coprolite <3642>	Coprolite <3492>	Coprolite <4029>
	8	9	10	11	12
A	0.108	0.064	0.046	0.056	1.476
B	0.115	0.059	0.045	0.045	0.041
C	0.059	0.051	0.043	0.049	0.049
D	0.045	0.036	0.044	0.042	0.048
E	0.071	0.044	0.043	0.049	0.045
F	0.04	0.045	0.044	0.044	0.045
G	0.111	0.047	0.045	0.047	0.052
H	0.829	0.427	1.199	0.78	0.29

B.2 ELISA Absorbance Values from Arlon

Table B2.1: Absorbance values from *Cryptosporidium* ELISA (test 1 left and test 2 right) for the Arlon latrine sample. Positive samples are highlighted in yellow. Positive and negative control are in red boxes.

	Arlon	Other Sample
	5	12
A	0.069	1.472
B	0.091	0.048
C	0.099	
D	0.141	
E	0.062	
F	0.053	
G	0.087	
H	0.064	

	Arlon	Other Sample
	5	12
A	0.063	1.645
B	0.06	0.051
C	0.06	
D	0.064	
E	0.063	
F	0.06	
G	0.064	
H	0.059	

Table B2.2: Absorbance values from *Entamoeba* ELISA (test 1 left and test 2 right) for the Arlon latrine sample. Positive samples are highlighted in yellow. Positive and negative control are in red boxes.

	Arlon	Other Sample
	5	12
A	0.08	1.731
B	0.088	0.042
C	0.059	
D	0.062	
E	0.064	
F	0.04	
G	0.055	
H	0.044	

	Arlon	Other Sample
	5	12
A	0.047	2.298
B	0.045	0.033
C	0.041	
D	0.046	
E	0.04	
F	0.037	
G	0.047	
H	0.047	

Table B2.3: Absorbance values from *Giardia* ELISA (test 1 left and test 2 right) for the Arlon latrine sample. Positive samples are highlighted in yellow. Positive and negative control are in red boxes.

	Arlon	Other Sample
	5	12
A	0.116	2.528
B	0.088	0.053
C	0.069	
D	0.175	
E	0.114	
F	0.087	
G	0.084	
H	0.071	

	Arlon	Other Sample
	5	12
A	0.042	1.476
B	0.043	0.041
C	0.043	
D	0.032	
E	0.033	
F	0.033	
G	0.038	
H	0.037	

B.3 ELISA Absorbance Values from Viminacium

B3.1 Absorbance values from *Cryptosporidium* ELISA (test 1 left and test 2 right) for the Viminacium coprolite sample. Positive samples are highlighted in yellow. Positive and negative control are in red boxes.

	Viminacium Mortar/Pestle	Viminacium HCl
	11	12
A	0.036	0.429
B	0.034	0.036
C	0.033	0.042
D	0.033	0.035
E	0.032	0.036
F	0.034	0.034
G	0.037	0.034
H	0.034	0.033

	Viminacium Mortar/Pestle	Other Sample
	11	12
A	0.043	0.956
B	0.031	0.036
C	0.036	
D	0.03	
E	0.033	
F	0.032	
G	0.034	
H	0.033	

B3.2 Absorbance values from *Entamoeba* ELISA (test 1 left and test 2 right) for the Viminacium coprolite sample. Positive samples are highlighted in yellow. Positive and negative control are in red boxes.

	Viminacium Mortar/Pestle	Viminacium HCl
	11	12
A	0.033	1.971
B	0.03	0.035
C	0.034	0.034
D	0.034	0.031
E	0.032	0.032
F	0.033	0.034
G	0.032	0.036
H	0.033	0.033

	Viminacium Mortar/Pestle	Other Sample
	11	12
A	0.031	1.978
B	0.032	0.031
C	0.032	
D	0.035	
E	0.035	
F	0.032	
G	0.034	
H	0.029	

B3.2 Absorbance values from *Giardia* ELISA (test 1 left and test 2 right) for the Viminacium coprolite sample. Positive samples are highlighted in yellow. Positive and negative control are in red boxes.

	Viminacium Mortar/Pestle	Viminacium HCl
	11	12
A	0.032	1.103
B	0.032	0.032
C	0.033	0.033
D	0.032	0.033
E	0.029	0.03
F	0.032	0.04
G	0.033	0.033
H	0.032	0.034

	Viminacium Mortar/Pestle	Other Sample
	11	12
A	0.031	2.198
B	0.049	0.032
C	0.031	
D	0.032	
E	0.031	
F	0.035	
G	0.032	
H	0.033	

B.4 ELISA Absorbance Values from Ephesus

B4.1 Absorbance values from *Cryptosporidium* ELISA (test 1 left and test 2 right) for the Ephesus public latrine samples. Positive samples are highlighted in yellow. Positive and negative control are in red boxes.

	Ephesus 4-6cm	Ephesus 9-11cm	Other Sample
	6	7	12
A	0.033	0.034	0.352
B	0.033	0.038	0.034
C	0.042	0.042	
D	0.043	0.043	
E	0.033	0.036	
F	0.033	0.034	
G	0.034	0.032	
H	0.033	0.037	

	Ephesus 9-11cm	Other Sample
	9	12
A	0.038	0.429
B	0.033	0.036
C	0.033	
D	0.03	
E	0.034	
F	0.042	
G	0.042	
H	0.033	

B4.2 Absorbance values from *Entamoeba* ELISA (test 1 left and test 2 right) for the Ephesus public latrine samples. Positive samples are highlighted in yellow. Positive and negative control are in red boxes.

	Ephesus 4-6cm	Ephesus 9-11cm	Other Sample
	6	7	12
A	0.038	0.034	0.501
B	0.034	0.033	0.054
C	0.034	0.036	
D	0.036	0.034	
E	0.033	0.038	
F	0.037	0.034	
G	0.034	0.036	
H	0.042	0.037	

	Ephesus 9-11cm	Other Sample
	9	12
A	0.037	1.971
B	0.035	0.035
C	0.034	
D	0.048	
E	0.033	
F	0.034	
G	0.033	
H	0.034	

B4.3 Absorbance values from *Giardia* ELISA (test 1 left and test 2 right) for the Ephesus public latrine samples. Positive samples are highlighted in yellow. Positive and negative control are in red boxes.

	Ephesus 4-6cm	Ephesus 9-11cm	Other Sample
	6	7	12
A	0.041	0.045	0.852
B	0.044	0.045	0.048
C	0.046	0.048	
D	0.05	0.046	
E	0.042	0.047	
F	0.046	0.046	
G	0.046	0.047	
H	0.046	0.036	

	Ephesus 9-11cm	Other Sample
	9	12
A	0.033	1.103
B	0.032	0.032
C	0.032	
D	0.034	
E	0.034	
F	0.033	
G	0.032	
H	0.032	

B.5 ELISA Absorbance Values from Sardis

B5.1 Absorbance values from *Cryptosporidium* ELISA (test 1) for the Sardis drain samples. Positive samples are highlighted in yellow. Positive and negative control are in red boxes.

	Sardis Basket 66 Bottom	Sardis Basket 67	Sardis Basket 59	Other Sample
	7	8	10	12
A	0.037	0.04	0.039	0.429
B	0.034	0.035	0.036	0.036
C	0.033	0.032	0.033	
D	0.033	0.034	0.033	
E	0.033	0.033	0.033	
F	0.033	0.044	0.039	
G	0.033	0.034	0.035	
H	0.034	0.033	0.035	

B5.2 Absorbance values from *Cryptosporidium* ELISA (test 2) for the Sardis drain samples. Positive samples are highlighted in yellow. Positive and negative control are in red boxes.

	Sardis Basket 59	Sardis Basket 66 Bottom	Sardis Basket 66 Top	Sardis Basket 67	Other Sample
	5	6	8	9	12
A	0.041	0.036	0.032	0.032	0.956
B	0.046	0.041	0.034	0.029	0.036
C	0.037	0.036	0.034	0.031	
D	0.038	0.041	0.039	0.034	
E	0.039	0.032	0.032	0.032	
F	0.042	0.031	0.033	0.031	
G	0.041	0.031	0.033	0.033	
H	0.033	0.036	0.034	0.032	

B5.3 Absorbance values from *Entamoeba* ELISA (test 1) for the Sardis drain samples. Positive samples are highlighted in yellow. Positive and negative control are in red boxes.

	Sardis Basket 66 Bottom	Sardis Basket 67	Sardis Basket 59	Other Sample
	7	8	10	12
A	0.035	0.033	0.034	1.971
B	0.047	0.035	0.038	0.035
C	0.034	0.034	0.034	
D	0.037	0.038	0.035	
E	0.059	0.036	0.061	
F	0.038	0.032	0.036	
G	0.036	0.037	0.035	
H	0.044	0.032	0.035	

B5.4 Absorbance values from *Entamoeba* ELISA (test 2) for the Sardis drain samples. Positive samples are highlighted in yellow. Positive and negative control are in red boxes.

	Sardis Basket 59	Sardis Basket 66 Bottom	Sardis Basket 66 Top	Sardis Basket 67	Other Sample
	5	6	8	9	12
A	0.034	0.032	0.032	0.035	1.978
B	0.035	0.033	0.033	0.033	0.031
C	0.035	0.034	0.032	0.034	
D	0.038	0.035	0.033	0.036	
E	0.038	0.033	0.033	0.033	
F	0.038	0.034	0.034	0.034	
G	0.035	0.032	0.032	0.033	
H	0.037	0.033	0.035	0.028	

B5.5 Absorbance values from *Giardia* ELISA (test 1) for the Sardis drain samples. Positive samples are highlighted in yellow. Positive and negative control are in red boxes.

	Sardis Basket 66 Bottom	Sardis Basket 67	Sardis Basket 59	Other Sample
	7	8	10	12
A	0.033	0.035	0.034	1.103
B	0.033	0.031	0.033	0.032
C	0.032	0.033	0.034	
D	0.032	0.033	0.034	
E	0.034	0.035	0.033	
F	0.032	0.032	0.034	
G	0.033	0.032	0.038	
H	0.033	0.034	0.034	

B5.6 Absorbance values from *Giardia* ELISA (test 2) for the Sardis drain samples. Positive samples are highlighted in yellow. Positive and negative control are in red boxes.

	Sardis Basket 59	Sardis Basket 66 Bottom	Sardis Basket 66 Top	Sardis Basket 67	Other Sample
	5	6	8	9	12
A	0.032	0.031	0.031	0.031	2.198
B	0.036	0.037	0.034	0.034	0.032
C	0.035	0.032	0.032	0.029	
D	0.034	0.033	0.034	0.031	
E	0.033	0.032	0.031	0.031	
F	0.037	0.032	0.032	0.05	
G	0.031	0.034	0.037	0.032	
H	0.063	0.033	0.032	0.033	

B.6 Palaeoparasitology in Romano-British Sites

Table B6.1: Previous parasitological studies of Romano-British sites used for analysis in section 5.4.

Parasite Species	Site name	Site type	Sample Type	Date (CE)	Reference
Ascaris sp.	Ambleside	Military	pit	1-400	Jones, 1985
Trichuris sp.	Ambleside	Military	pit	1-400	
Ascaris sp.	Bearsden	Military	sewer/drain	142-158	Knights et al., 1983
Trichuris sp.	Bearsden	Military	sewer/drain	142-158	
Ascaris sp.	Carlisle	Military	occupation layer	1-300	Jones and Hutchison, 1991
Fasciola sp.	Carlisle	Military	occupation layer	1-300	
Trichuris sp.	Carlisle	Military	occupation layer	1-300	
Ascaris sp.	Church Street Sewer, York	Urban	sewer/drain	1-500	Wilson and Rackham, 1976
Trichuris sp.	Church Street Sewer, York	Urban	sewer/drain	1-500	
Ascaris sp.	Leicester	Urban	cesspit/latrine	1-200	Boyer, 1999
Fasciola sp.	Leicester	Urban	cesspit/latrine	1-200	
Trichuris sp.	Leicester	Urban	cesspit/latrine	1-200	
Trichuris sp.	Lincoln Waterside NW	Urban	occupation layer	301-400	Carrott et al., 1995
Dicrocoelium sp.	London, 15-35 Copthall Ave	Urban	ditch	101-400	De Moulins, 1990
Trichuris sp.	London, 15-35 Copthall Ave	Urban	ditch	101-400	
Ascaris sp.	London, Hibernia Wharf	Urban	well	1-200	Rouffignac, 1985
Diphyllobothrium sp.	London, Hibernia Wharf	Urban	well	1-200	
Taenia sp.	London, Hibernia Wharf	Urban	well	1-200	
Trichuris sp.	London, Hibernia Wharf	Urban	well	1-200	
Ascaris sp.	Owslebury, Winchester	Rural	pit	Roman	Pike, 1968
Dicrocoelium sp.	Owslebury, Winchester	Rural	pit	Roman	
Trichuris sp.	Owslebury, Winchester	Rural	pit	Roman	
Ascaris sp.	Poundbury, Dorset	Military	burial	Roman	Jones, 1987
Trichuris sp.	Poundbury, Dorset	Military	burial	Roman	
Ascaris sp.	Westsmithfield, London	Urban	burial	Roman	New data

B.7 Statistics for Temporal Changes

These are the statistical tests used for section 6.1.

To determine if the proportions of positive sites in different time periods are independent of time period a Chi-square test for independence was used. For this statistic the expected values are calculated using the total number of sites and the X^2 statistic is calculated as per the formula $X^2 = \sum \frac{(O-E)^2}{E}$, where O = observed value and E = expected value. The expected value is calculated as $\frac{(total\ for\ time\ period)\ x\ (total\ for\ column)}{total\ number\ of\ sites\ in\ cell}$. The function 'chi2_contingency' in the statistics module of SciPy running with Python3 was used and the for the calculations and screen shots of each script are provided.

B7.1. *Trichuris* sp.

	Positive Sites	Negative Sites	Total
Pre-Roman	25	21	46
Roman	38	24	62
Medieval	37	18	55
Total	100	63	163

```
In [39]: #create array of Pre-Roman and Roman Trichuris frequencies
         TrichurisPR=numpy.array([[25,21],[38,24]])
         #calculate Chi-square test statistic, output is (X2, p-value, degrees of freedom, array of expected values)
         chi2_contingency(whipwormPR, correction=False)

Out[39]: (0.5236625926667999, 0.4692834921730814, 1, array([[26.83333333, 19.16666667],
                [36.16666667, 25.83333333]]))

In [38]: #create array of Roman and Medieval Trichuris frequencies
         TrichurisRM=numpy.array([[38,24],[37,18]])
         #calculate Chi-square test statistic, output is (X2, p-value, degrees of freedom, array of expected values)
         chi2_contingency(whipwormRM, correction=False)

Out[38]: (0.45329534981147934, 0.5007741853186212, 1, array([[39.74358974, 22.25641026],
                [35.25641026, 19.74358974]]))
```

For comparison between Pre-Roman and Roman proportions, X^2=0.5237, p=0.4693, dof=1

With $\alpha = 0.05$; accept H_0 because X^2=0.5237 < 3.841. Time period and number of sites with whipworm are independent.

For comparison between Roman and Medieval proportions, X^2=0.4533, p=0.5008, dof=1

With $\alpha = 0.05$; accept H_0 because X^2=0.4533 < 3.841. Time period and number of sites with whipworm are independent.

B7.2. *Ascaris* sp.

	Positive Sites	Negative Sites	Total
Pre-Roman	14	32	46
Roman	40	22	62
Medieval	41	14	55
Total	95	68	163

```
In [37]: #create array of Pre-Roman and Roman Ascaris frequencies
         AscarisPR=numpy.array([[14,32],[40,22]])
         #calculate Chi-square test statistic, output is (X2, p-value, degrees of freedom, array of expected values)
         chi2_contingency(AscarisPR, correction=False)

Out[37]: (12.269284712482468, 0.0004604747348062683, 1, array([[23., 23.],
                [31., 31.]]))

In [36]: #create array of Roman and Medieval Ascaris frequencies
         AscarisRM=numpy.array([[40,22],[41,14]])
         #calculate Chi-square test statistic, output is (X2, p-value, degrees of freedom, array of expected values)
         chi2_contingency(AscarisRM, correction=False)

Out[36]: (1.3762463343108498, 0.2407415693494686, 1, array([[42.92307692, 19.07692308],
                [38.07692308, 16.92307692]]))
```

*For comparison between Pre-Roman and Roman proportions, X^2=12.2693, p=0.0005, dof=1

With $\alpha = 0.05$; reject H_0 because X^2=12.2693 > 3.841. Time period and number of sites with roundworm are not independent.

For comparison between Roman and Medieval proportions, X^2=1.3762, p=0.2407, dof=1

With $\alpha = 0.05$; accept H_0 because X^2=1.3762 < 3.841. Time period and number of sites with roundworm are independent.

B7.3. *Dicrocoelium* sp.

	Positive Sites	Negative Sites	Total
Pre-Roman	13	33	46
Roman	8	54	62
Medieval	12	43	55
Total	33	130	163

```
In [40]: #create array of Pre-Roman and Roman Dicrocoelium frequencies
         DicroPR=numpy.array([[13,33],[8,54]])
         #calculate Chi-square test statistic, output is (X2, p-value, degrees of freedom, array of expected values)
         chi2_contingency(DicroPR, correction=False)

Out[40]: (3.976343625422312, 0.046143625439484165, 1, array([[ 8.94444444, 37.05555556],
                [12.05555556, 49.94444444]]))

In [41]: #create array of Roman and Medieval Dicrocoelium frequencies
         DicroRM=numpy.array([[8,54],[12,43]])
         #calculate Chi-square test statistic, output is (X2, p-value, degrees of freedom, array of expected values)
         chi2_contingency(DicroRM, correction=False)

Out[41]: (1.6344698733258753, 0.2010859153157088, 1, array([[10.5982906, 51.4017094],
                [ 9.4017094, 45.5982906]]))
```

*For comparison between Pre-Roman and Roman proportions, X^2=3.9763, p=0.0461, dof=1

With $\alpha = 0.05$; reject H_0 because X^2=3.9763 > 3.841. Time period and number of sites with *Dicrocoelium* sp. are not independent.

For comparison between Roman and Medieval proportions, X^2=1.6345, p=0.2011, dof=1

With $\alpha = 0.05$; accept H_0 because X^2=1.6345 < 3.841. Time period and number of sites with *Dicrocoelium* sp. are independent.

B7.4. *Fasciola* sp.

	Positive Sites	Negative Sites	Total
Pre-Roman	18	28	46
Roman	8	54	62
Medieval	6	49	55
Total	32	131	163

```
In [42]: #create array of Pre-Roman and Roman Fasciola frequencies
         FasciolaPR=numpy.array([[18,28],[8,54]])
         #calculate Chi-square test statistic, output is (X2, p-value, degrees of freedom, array of expected values)
         chi2_contingency(FasciolaPR, correction=False)

Out[42]: (9.937799483723607,
          0.0016191899499580203,
          1,
          array([[11.07407407, 34.92592593],
                 [14.92592593, 47.07407407]]))

In [43]: #create array of Roman and Medieval Fasciola frequencies
         FasciolaRM=numpy.array([[8,54],[6,49]])
         #calculate Chi-square test statistic, output is (X2, p-value, degrees of freedom, array of expected values)
         chi2_contingency(FasciolaRM, correction=False)

Out[43]: (0.11002314315812574, 0.7401177891913779, 1, array([[ 7.41880342, 54.58119658],
                 [ 6.58119658, 48.41880342]]))
```

*For comparison between Pre-Roman and Roman proportions, X^2=9.9378, p=0.0016, dof=1

With $\alpha = 0.05$; reject H_0 because X^2=9.9378 > 3.841. Time period and number of sites with *Fasciola* sp. are not independent.

For comparison between Roman and Medieval proportions, X^2=0.1100, p=0.7401, dof=1

With $\alpha = 0.05$; accept H_0 because X^2=0.1100 < 3.841. Time period and number of sites with *Fasciola* sp. are independent.

B7.5. *Taenia* sp.

	Positive Sites	Negative Sites	Total
Pre-Roman	14	32	46
Roman	7	55	62
Medieval	11	44	55
Total	32	131	163

```
In [44]: #create array of Pre-Roman and Roman Taenia frequencies
         TaeniaPR=numpy.array([[14,32],[7,55]])
         #calculate Chi-square test statistic, output is (X2, p-value, degrees of freedom, array of expected values)
         chi2_contingency(TaeniaPR, correction=False)

Out[44]: (6.179039512501814, 0.012927243186140826, 1, array([[ 8.94444444, 37.05555556],
                [12.05555556, 49.94444444]]))

In [45]: #create array of Roman and Medieval Taenia frequencies
         TaeniaRM=numpy.array([[7,55],[11,44]])
         #calculate Chi-square test statistic, output is (X2, p-value, degrees of freedom, array of expected values)
         chi2_contingency(TaeniaRM, correction=False)

Out[45]: (1.6983870967741939, 0.19249904482143218, 1, array([[ 9.53846154, 52.46153846],
                [ 8.46153846, 46.53846154]]))
```

*For comparison between Pre-Roman and Roman proportions, X^2=6.1790, p=0.0129, dof=1

With $\alpha = 0.05$; reject H_0 because X^2=6.1790 > 3.841. Time period and number of sites with *Taenia* sp. are not independent.

For comparison between Roman and Medieval proportions, X^2=1.6984, p=0.1925, dof=1

With $\alpha = 0.05$; accept H_0 because X^2=1.6984 < 3.841. Time period and number of sites with *Taenia* sp. are independent.

B7.6. *Diphyllobothrium* sp.

	Positive Sites	Negative Sites	Total
Pre-Roman	16	30	46
Roman	6	56	62
Medieval	9	46	55
Total	31	132	163

```
In [46]: #create array of Pre-Roman and Roman Diphyllobothrium frequencies
         DiphyPR=numpy.array([[16,30],[6,56]])
         #calculate Chi-square test statistic, output is (X2, p-value, degrees of freedom, array of expected values)
         chi2_contingency(DiphyPR, correction=False)

Out[46]: (10.26075095849061,
          0.0013589035362367315,
          1,
          array([[ 9.37037037, 36.62962963],
                 [12.62962963, 49.37037037]]))

In [47]: #create array of Roman and Medieval Diphyllobothrium frequencies
         DiphyRM=numpy.array([[6,56],[9,46]])
         #calculate Chi-square test statistic, output is (X2, p-value, degrees of freedom, array of expected values)
         chi2_contingency(DiphyRM, correction=False)

Out[47]: (1.1657616008280147, 0.2802738319889655, 1, array([[ 7.94871795, 54.05128205],
                 [ 7.05128205, 47.94871795]]))
```

*For comparison between Pre-Roman and Roman proportions, X^2=10.2608, p=0.0014, dof=1

With $\alpha = 0.05$; reject H_0 because X^2=10.2608 > 3.841. Time period and number of sites with *Diphyllobothrium* sp. are not independent.

For comparison between Roman and Medieval proportions, X^2=1.1658 p=0.2803, dof=1

With $\alpha = 0.05$; accept H_0 because X^2=1.1658 < 3.841. Time period and number of sites with *Diphyllobothrium* sp. are independent.

B7.7. *Capillaria* sp.

	Positive Sites	Negative Sites	Total
Pre-Roman	9	37	46
Roman	6	56	62
Medieval	2	53	55
Total	17	146	163

```
In [3]: #create array of Pre-Roman and Roman Capillaria frequencies
        CapPR=numpy.array([[9,37],[6,56]])
        #calculate Chi-square test statistic, output is (X2, p-value, degrees of freedom, array of expected values)
        chi2_contingency(CapPR, correction=False)

Out[3]: (2.1587295842193366, 0.14176185393345736, 1, array([[ 6.38888889, 39.61111111],
                [ 8.61111111, 53.38888889]]))

In [4]: #create array of Roman and Medieval Capillaria frequencies
        CapRM=numpy.array([[6,56],[2,53]])
        #calculate Chi-square test statistic, output is (X2, p-value, degrees of freedom, array of expected values)
        chi2_contingency(CapRM, correction=False)

Out[4]: (1.6697422583335575, 0.1962930603865599, 1, array([[ 4.23931624, 57.76068376],
                [ 3.76068376, 51.23931624]]))
```

For comparison between Pre-Roman and Roman proportions, X^2=2.1587, p=0.1418, dof=1

With $\alpha = 0.05$; accept H_0 because X^2=2.1587 < 3.841. Time period and number of sites with *Capillaria* sp. are independent.

For comparison between Roman and Medieval proportions, X^2=1.6697 p=0.1963, dof=1

With $\alpha = 0.05$; accept H_0 because X^2=1.6697 < 3.841. Time period and number of sites with *Capillaria* sp. are independent.