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**Keywords:** latrine; medieval; Middle East; migration; palaeoparasitology; pilgrimage.

## **Abstract**

The aim of this research is to determine which parasites were present in a mediaeval latrine from the old city of Jerusalem. This latrine contains fragments of pottery from the Middle East and also from Italy, suggesting links of some kind with Europe. Excavation identified two separate entry chutes emptying in a shared cesspool. Radiocarbon dating and pottery analysis is compatible with a date of use in the late fifteenth century and early sixteenth century. Twelve coprolites (preserved stool) and mixed cesspool sediment were analysed with light microscopy and enzyme-linked immunosorbent assay (ELISA). Six species of intestinal parasites were identified. These were the helminths *Ascaris lumbricoides* (roundworm), *Trichuris trichiura* (whipworm), *Taenia* sp. (beef/pork/asiatic tapeworm) *Diphyllobothrium* sp. (fish tapeworm), and two protozoa that can cause dysentery (*Entamoeba histolytica* and *Giardia duodenalis*). While roundworm and whipworm were found in every sample, the other parasite species were present in only one or two samples each, suggesting that only a minority of those using the latrine were infected with those species. The role of Jerusalem as a site for long distance trade, migration or pilgrimage is considered when interpreting the Italian pottery and the parasites present, especially *E. histolytica* and *Diphyllobothrium* sp.

## **1. Introduction**

Jerusalem has long been known as a crossroads between Europe, Africa and Asia due to its geographic location. Like many cities in the Middle East, in the medieval period its population was comprised of people of different religious beliefs including Muslims, Christians and Jews (Little, 1989; Badr, 2005; Braude and Lewis, 1982). The cultural and religious complexity of the local population of the city has resulted in the differing attitudes to diet and food hygiene noted in these three religions. However, its role as a city holy in Judaism, Christianity and Islam made it a destination for so many more travellers. As well as the merchants who came to trade, pilgrims travelled long distances to pray at sites that they held precious in their traditions (Janin, 2002; Prescott, 1954). This complex mixture of religions and cultures coupled with the presence of long distance travellers makes Jerusalem an ideal site for research into health in the past, and in particular how past infectious diseases may have been spread by those travellers.

The aim of this study is to analyse samples from the contents of a medieval cesspool from the Christian quarter of the old city of Jerusalem, in order to determine if intestinal parasites were present. This will allow us not only to better understand the health of those who lived in the Christian quarter of the city at that time, but also to look for signs of infectious diseases that were not endemic to the Jerusalem region. This should highlight the presence of long distance travellers who had taken their parasites with them as they journeyed to Jerusalem.

## **2. Materials**

In 1996 rescue excavations were carried out in the courtyard of the Spanish School (Colegio del Pilar) in the Christian Quarter of Jerusalem. This lies a short distance to the north of the Church of the Holy Sepulchre (Fig.1). Three small test pits were opened, in one of which a stone-built cesspit was discovered and partly excavated. The cesspit had a vaulted roof, stone-built walls, an earth floor and two entry chutes on opposing sides (Fig.2). As it was only partly excavated the full dimensions are unknown, but its height was noted to be about 2.5m to the top of the vault and width estimated at about 2.40m. A fragment of charcoal from the cesspool sediment (level 8) was sent for AMS radiocarbon dating at the Oxford Radiocarbon Accelerator Unit (ORAU), UK and the calibrated date indicated the tree dated from 1304-1413AD with 95% probability (Fig. 3). This falls within the Mamluk Period (1250-1516 AD). The pottery recovered within the cesspool suggested the use of the cesspit in the later 1400s. Since wood is often stored or used in different ways before eventually being burnt and forming charcoal, these results together would be compatible with use of the cesspool during the second half of the fifteenth century and early sixteenth century. At this time Jerusalem was under the jurisdiction of the viceroy of Damascus (Little, 1989). Pottery in the cesspool includes both local ware and imports from northern Italy (Fig. 4), and the function as a cesspit is supported by the presence of numerous chamber pots.

### **3. Methods**

Sediment from level 8 of the cesspool was sieved and 12 lightly mineralized coprolites (pieces of stool) were identified. Further sediment was also stored. These samples recently underwent analysis for evidence for ancient parasites. 0.2g subsamples of each sample was weighed and taken for disaggregation using one of two techniques, namely 0.5% trisodium phosphate and distilled water (Anastasiou and Mitchell, 2013). This allowed a comparison of

whether any extra species were identified using either technique. After one hour the samples had been fully disaggregated. This suspension was passed through a column of sequential micro-sieves with mesh size of 300 $\mu$ m, 160 $\mu$ m and 20 $\mu$ m. Most parasite eggs have dimensions of 25-150 $\mu$ m so will be trapped on the 20 $\mu$ m sieve (Bouchet et al., 1999; Bouchet et al. 2003). After washing the sediment from the 20 $\mu$ m sieve the suspension was centrifuged to concentrate the sediment, excess water removed, and glycerol added prior to mounting on slides to ensure a clear view of any parasite eggs present. In order to identify the parasite remains the slides were examined using digital light microscopy. Egg counts per gram of soil can be determined using a number of methods (Reinhard et al. 1986), but we determined them by counting the number of eggs in the entire 0.2g sample and multiplying the eggs present by five. This has the advantage of avoiding the need for lycopodium spores, and also avoids the potential bias of taking an aliquot of disaggregated fluid to represent the larger original sample when the eggs may not be uniformly distributed throughout the disaggregation fluid. Parasite eggs were identified based upon their shape, size, colour, and special characteristics when compared with standard parasitology sources (Garcia, 2009; Gunn and Pitt, 2012).

Disaggregated latrine soil that passed through the 20 $\mu$ m sieve was collected and analysed for evidence of the protozoal organisms that cause dysentery. Cysts of *Giardia duodenalis*, *Entamoeba histolytica* and *Cryptosporidium parvum* measure around 5-20 $\mu$ m in size (Garcia, 2009). Samples of this fluid were tested with Enzyme Linked Immunosorbent Assay (ELISA) kits specifically designed by TECHLAB<sup>®</sup> to detect these species of organisms in faecal specimens (<http://www.techlab.com>). The test uses monoclonal antibody-peroxidase conjugate specific for proteins uniquely secreted by these organisms. This makes the test more sensitive and specific than microscopy for protozoal cysts (Sharp et al., 2001).

## 4. Results

### 4.1. Helminths

All 12 coprolites and also the sediment from the cesspool were positive for the eggs of both whipworm (*Trichuris trichiura*) (Fig.5) and roundworm (*Ascaris lumbricoides*) (Fig.6) and these two species were the most common eggs found (Table 1 and 2). Whipworm was identified by its lemon shape, brown colour, polar plugs, and its dimensions. Roundworm was identified by its oval shape, brown colour, dimensions and mammillated coat. Two coprolites (no.5 and no.10) were positive in low numbers (3 eggs) for the eggs of *Taenia sp.* tapeworm (potentially *T. solium*, *T. saginata*, or *T. asiatica*), which appear identical at this stage of their life cycle (Fig.7). These were identified by their round shape, thick wall with striations, brown colour and dimensions. One coprolite (no. 11) was positive in low numbers (6 eggs) for fish tapeworm (*Diphyllobothrium sp.*) (Fig.8). This was identified by the oval shape, absence of colour, operculum at one pole, and dimensions that distinguish it from *Spirometra sp.* and other operculated eggs. No extra species of parasite were identified in the samples disaggregated in trisodium phosphate when compared with the matched sample disaggregated in distilled water, suggesting that neither technique was superior to the other in this study.

### 4.2 Protozoa

ELISA analysis tested for the single celled protozoal parasites that can cause dysentery, specifically *Entamoeba histolytica*, *Giardia duodenalis* and *Cryptosporidium parvum*. The cesspool sediment was positive for *Giardia duodenalis* (1 sample out of 6 tested) but the coprolites tested were negative for giardia. For a positive giardia result, the ELISA reading

must be greater than 0.150 and the positive sample gave a reading of 0.699. Analysis for *Entamoeba histolytica* was positive for coprolite no.2 (2 samples out of 8 tested) but negative for the sediment and the other 11 coprolites. For a positive *Entamoeba histolytica II* test the positive test wells must have a reading at least 0.050 higher than the negative control well value on the test kit (0.035 on this occasion). The positive values for coprolite no.2 were 0.111 and 0.095.

## **5. Discussion**

Analysis of this cesspool from fifteenth century Jerusalem has identified the eggs or cysts of six different parasites. We believe all the parasites to have come from human faecal matter as this was a latrine from a town house, all the parasites are compatible in size and shape with those that infect humans, some of the species are unique to humans, and no species unique to animals were present. In order to interpret the significance of these discoveries, we will discuss what we might expect to find in a latrine from the region at that time, and then go on to explain why some of these finds are of particular interest.

Past parasite research at sites in the Levant and Iran has identified the presence of whipworm, roundworm, Taenia tapeworm, fasciola liver fluke, pinworm, hydatid worm, lancet liver fluke, fish tapeworm, and both *Entamoeba histolytica* and *Giardia duodenalis* protozoa (Cahill et al., 1991; Harter et al., 2004; Mitchell and Tepper, 2007; Mitchell et al., 2008; Mitchell et al., 2011; Nezamabadi et al. 2013a; Nezamabadi et al. 2013b; Zias et al., 2006). It is quite likely that the whipworm, roundworm, beef/pork tapeworm and liver fluke had become endemic to the region long before the medieval period, as they had been found in Iron Age, Roman and Byzantine contexts and are thought to have been infecting humans throughout our evolution in Africa (Mitchell, 2013). Adult whipworms are 3-5cm long but roundworms are much

larger at 20-30cm long (Garcia, 2009). Whipworm and roundworm are spread by the faecal contamination of food, either by the use of faeces as a crop fertiliser or when preparing meals with unwashed hands. They are thought to have become progressively more common in Europe and the Middle East following the development of agriculture and sedentary life in towns and cities with limited sanitation (Reinhard et al., 2013).

However, some of the other species do not appear to have been endemic to the region. Both species of protozoa (*Entamoeba histolytica* and *Giardia duodenalis*) have only previously been identified in the region in crusader period latrines in the coastal city of Acre (Mitchell et al., 2008). The earliest known cases of *E. histolytica* and *G. duodenalis* have been identified in Europe (Goncalves et al 2002; Goncalves et al., 2004; Le Bailly and Bouchet, 2006).

Recent research has suggested that *E. histolytica* may have actually evolved in Europe before being spread to other regions of the world at a later date (Le Bailly and Bouchet, 2015). It is possible that *E. histolytica* spread to the Middle East at the time of the crusades and became endemic in the region from that time, hence the finding of that species in the Jerusalem cesspool. Another possibility is that a traveller from Europe brought the infection with them in their intestines, and used this cesspool during their stay in the city.

Fish tapeworm can reach 10m long in their human hosts, and coil around inside the intestines. It was common in northern Europe during the medieval period, but has been absent in medieval latrines from southern Europe and the Middle East outside the context of crusader latrines used by Europeans (Sianto et al., 2009; Mitchell et al. 2011; Yeh et al., 2014; Anastasiou, 2015). *Diphyllobothrium sp.* has been recently reported in Roman Period Caesarea and Be'er Sheva in Israel in a dissertation (Le Bailly and Bouchet, 2013), but other latrines and coprolites from the mainland Middle East dating from a range of time periods

have been negative for fish tapeworm (Anastasiou and Mitchell, 2015). It is not present in the region in modern times. This suggests either that it was quite rare in the Middle East in the past, or non-endemic and only present in travellers to the region from those parts of northern Europe where it was common. There are a number of potential reasons for this. It may be that the environment of the local lakes and rivers is not suited to the complex life cycle of the fish tapeworm which requires spending time in a number of specific intermediate hosts including copepods, small fresh water fish and potentially larger fish (Garcia, 2009: 354). Also, cuisine in the medieval Middle East typically involved cooking fish, and not eating it raw, smoked or pickled (Lewicka, 2011: 209-25). Cooking the fish kills the intermediate forms of the parasite and so prevents its transmission. The presence in the cesspool of the fragments of pottery made in Italy reinforces the links between Europe and the people using this latrine. In consequence, it is possible that the presence of fish tapeworm eggs in a coprolite from this cesspool indicates that a traveller from northern Europe infected with fish tapeworms may have used the latrine during their visit to Jerusalem.

It seems clear that some parasites were much more common than others among those using this latrine. Every sample was positive for the eggs of whipworm and roundworm, which suggests that infection with these parasites was widespread. Only one or two samples were positive for each of *Taenia* (beef/pork/Asiatic) tapeworm, fish tapeworm, and the protozoa that may cause dysentery (Table 1). This suggests that not all the coprolites originated from the same individual, as the parasite profile differed. It also suggests that these types of parasite were probably less common in those using this latrine, and were certainly not as ubiquitous as the faecal oral parasites (roundworm and whipworm).

Comparison of the egg count per gram of coprolite demonstrates quite a variation for both species, ranging from 20-2,200/g for roundworm, and from 15-300/g for whipworm (Table 2). The egg counts per gram in mineralised coprolites are not directly comparable with those of dried coprolites from a mummy or cave, as the minerals absorbed into the faeces from the cesspool after defecation will make it heavier than if dried. Roundworm generally produces around 200,000 eggs per day while whipworm generally produces much fewer, around 2,000-10,000 eggs per day (Bennett, 2006; Schmidt and Roberts, 2006: 397-9 and 431-3). In consequence, if the same number of roundworms and whipworms were present in an individual, we might expect to find 20-100 times more roundworm eggs than whipworm eggs in a sample. Table 2 shows that in each coprolite the number of roundworm eggs was higher than whipworm, but the proportions varied widely. In two coprolites (no.7 and 11) there were 20 times or more roundworm eggs than whipworm. However, in the other ten coprolites and in the sediment the proportions were between 1.3 and 9 times more roundworm than whipworm eggs. This may suggest that in most individuals, whipworm was more common than roundworm in this population. While egg counts will be influenced by the number of worms of each species present in the intestines of people using the cesspool, the day-to-day variation in egg production by intestinal parasites means that we cannot determine the worm numbers with any accuracy.

Study of Arabic textual sources help us to understand the kind of foods eaten in the Middle East during the Islamic Period. This information is important, as it helps us to understand the way the meat of certain animals may have spread parasites. Texts vary from descriptions of aristocratic courtly cuisine down to the kinds of food eaten by the poor (Rodinson, 2001; Zaouali, 2007: 10). Chicken and mutton were eaten frequently by the wealthy, while cattle and camel were described much less in courtly texts and seem to have been a food of the poor

(Lewicka, 2011: 173). Fish were described as being eaten along the Mediterranean coast and the great rivers such as Euphrates, Tigris and Nile, but rarely in the inland Syrian cities such as Damascus and Jerusalem (Perry, 2001). In Egypt it seems fish was mainly a food for the poor and it was always cooked, never eaten raw or smoked (Lewicka, 2011: 209-25).

Excavations of Mamluk period settlements in the Levant have shown the kinds of animals favoured in the diet. Analysis of Mamluk period levels at Horvat Shallale on Mount Carmel identified significant numbers of bones from cattle, sheep/goat, pig, chicken and deer (Horwitz, 2009). Such work highlights that despite the dominance of Islam in society, Christians were still allowed to farm and eat pigs. Since the latrine was located in the Christian quarter of Jerusalem, this means that the *Taenia sp.* eggs might have been from the pork tapeworm, beef tapeworm or Asiatic tapeworm, as we know the relevant intermediate host animals were consumed in the region during the Mamluk Period.

The impact of these different parasites upon the health of those infected would have depended upon which species were present in any one individual, and how many parasites were present. A light load of whipworm or roundworm would be unlikely to cause any symptoms or untoward health effects for that individual. However, a heavy load of whipworm and roundworm in children can lead to malnutrition, reduced intelligence and stunted growth (Garcia, 2009: 130-33; Gunn and Pitt, 2012: 127-9; Callender et al., 1998). Dysentery generally leads to diarrhoea with abdominal pain for a week or two, but a proportion of individuals may become seriously ill or die. Children and those who had not previously had dysentery are most at risk of death, which can result from dehydration, salt depletion, or septicaemia (Garcia, 2009: 274).

## **6. Conclusion**

The analysis of this fifteenth century latrine in Jerusalem provides a vivid glimpse of the infectious diseases suffered by the people who used it (Moore, 1981). It shows how parasitic worms spread by the faecal contamination of food (roundworm and whipworm) were quite common, with all samples tested being positive. However, other species of parasite were less common, so that only a minority of the population appeared to suffer with those. We have identified the presence of large tapeworms whose adult forms grow 10m long, and single celled protozoa that could multiply in huge numbers to inflame the intestinal lining and cause dysentery. Perhaps the most fascinating discovery has been the presence of protozoal species such as *Entamoeba histolytica* that are thought to have originated in Europe, and fish tapeworm that was common in northern Europe. Considering the context of a Levantine latrine, these results could be a consequence for the strong links with Europe at that time. The presence of Italian pottery in the latrine reinforces this hypothesis. One potential explanation is that the owners of this property were merchants from Jerusalem who travelled to Europe to do business and contracted parasites there as well as buying European pottery. Another option is that this was a hostel for European travellers such as merchants or pilgrims. While we can only suggest possible reasons as to why they made this journey to the Christian Quarter, it does seem that they brought with them unsuspecting hitch-hikers in their intestines.

## **Acknowledgements**

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## List of Figures

Figure 1. Map of Old City of Jerusalem, with site of the excavation marked by a star.

Reproduced courtesy of the École Biblique de Jérusalem.

Figure 2. Plan of the cesspool, courtesy of the École Biblique de Jérusalem.

Figure 3. AMS radiocarbon dating result for fragment of charcoal from the cesspool sediment.

Figure 4. *Graffita tarda*, bowl fragment, second half of the fifteenth century to first half of the sixteenth century, from the Veneto region of Italy. CP 157. Image: Jean-Baptiste Humbert.

Figure 5. *Trichuris trichiura* (whipworm) egg. Dimensions 49x24 µm. Black bar indicates 20µm.

Figure 6. *Ascaris lumbricoides* (roundworm) egg with its mammillated surface coat. Dimensions 60x44µm. Black bar indicates 20µm.

Figure 7. *Taenia sp.* (beef, pork or Asiatic tapeworm) egg. Dimensions 33x36µm. Black bar indicates 20µm.

Figure 8. *Diphyllobothrium sp.* (fish tapeworm) egg from coprolite 11. Dimensions 65x40µm. Arrow indicates the operculum. Black bar indicates 20µm.

Table 1. Species of parasite detected in the cesspool samples.

Table 2. Number of eggs per gram of *Ascaris lumbricoides* and *Trichuris trichiura* in coprolites and cesspool sediment.