



Parasite infection at the early farming community of Çatalhöyük

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Published in:
Antiquity

DOI:
[10.15184/aqy.2019.61](https://doi.org/10.15184/aqy.2019.61)

Publication date:
2019

Document version
Peer reviewed version

Citation for published version (APA):

Ledger, M. L., Anastasiou, E., Shillito, L-M., Mackay, H., Bull, I. D., Haddow, S. D., ... Mitchell, P. D. (2019). Parasite infection at the early farming community of Çatalhöyük. *Antiquity*, 93(369), 573-587. <https://doi.org/10.15184/aqy.2019.61>

Marissa L. Ledger, Evilena Anastasiou, Lisa-Marie Shillito, Helen Mackay, Ian D. Bull, Scott D. Haddow, Christopher Knüsel, Piers D. Mitchell (2019) Parasite infection at the early farming community of Çatalhöyük. *Antiquity* doi.org/10.15184/aqy.2019.61

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Received: 30 October 2018; Revised: 6 February 2019; Accepted: 26 February 2019

<LOCATION MAP, 6.5cm colour, place to left of abstract and wrap text around>

*The early village at Çatalhöyük (7100–6150 BC) provides important evidence for the Neolithic and Chalcolithic people of central Anatolia. This article reports on the use of lipid biomarker analysis to identify human coprolites from midden deposits, and microscopy to analyse these coprolites and soil samples from human burials. Whipworm (*Trichuris trichiura*) eggs are identified in two coprolites, but the pelvic soil samples are negative for parasites. Çatalhöyük is one of the earliest Eurasian sites to undergo palaeoparasitological analysis to date. The results inform how intestinal parasitic infection changed as humans modified their subsistence strategies from hunting and gathering to settled farming.*

Keywords: Anatolia, Çatalhöyük, Neolithic, palaeoparasitology, coprolite

Introduction

As human societies shifted from hunting and gathering to farming and herding, it is thought that their risk of contracting certain diseases also changed (Cohen & Armelagos 1984; Harper & Armelagos 2010; Reinhard *et al.* 2013; Mitchell 2015a). The Near East is the first of the independent centres where agriculture arose. Barley and wheat were domesticated in the Tigris-Euphrates basin, and subsequently, increasingly large settlements developed across the Fertile Crescent approximately 9000 years ago (Armelagos & Harper 2005; Brown *et al.* 2009). The domestication of crops and the rise of farming and herding necessitated and supported a sedentary lifestyle. Increases in food availability facilitated population expansion, resulting in the first epidemiological transition—the characteristic rise in infectious diseases associated with increased population size and mobility (Armelagos & Harper 2005). New lifeways during the Neolithic probably affected infectious disease transmission in many ways, such as through the accumulation of human waste, the alteration of plant and animal environments, increasing population size and higher population density. It has been argued that intestinal diseases, including parasitic infection, may also have altered at this time. For example, with a changing environment, the differing lifecycles of parasite species may have facilitated the spread of some parasites among humans, while hampering the spread of others (Mitchell 2013).

The three Mediterranean Neolithic period sites so far subjected to parasitological analysis have revealed a broad range of parasite species. In Neolithic Cyprus, pelvic soil from 11 burials at Shillourokambos (8300–7000 BC) and Khirokitia (7000–6000 BC) contained the eggs of the soil-transmitted helminths (geohelminths), that is roundworm and whipworm, and species that spend part of their lifecycle in cattle, pigs and sheep: namely, *Taenia* sp. tapeworm and *Fasciola* sp. liver fluke (Harter-Lailheugue *et al.* 2005). Whipworm, roundworm and pinworm were identified at the Neolithic village of La Draga at Lake Banyoles in Spain (5320–4980 BC), along with species that spend part of their lifecycle in cattle, fish, rodents and ants: namely, *Taenia saginata*, fish tapeworm (*Diphyllobothrium* sp.), *Capillaria* sp. and Lancet liver fluke (*Dicrocoelium dendriticum*) (Maicher *et al.* 2017). While parasite studies have been undertaken at Neolithic sites in Europe (Anastasiou 2015), very little research has investigated the species of intestinal parasites present in the early large village agglomerations of the Near East.

The Neolithic East Mound of Çatalhöyük in south-central Anatolia (Figure 1) was occupied continuously for more than 1000 years from 7100–6000 cal BC (Bayliss *et al.* 2015), during the late Pre-Pottery Neolithic. On the nearby West Mound, occupation of the site continued

into the Chalcolithic period until the middle of the sixth millennium BC (Orton *et al.* 2018). Throughout the occupation of the East Mound, the settlement pattern gradually changed from a dense agglomeration, where people lived in buildings that were constructed directly adjacent to each other with no space for streets in between, to a more open layout during the later occupation phase (Hodder 2014). Throughout the settlement's lifespan, buildings were clustered in neighbourhoods, often showing long-term continuity of occupation, with multiple episodes of rebuilding in the same location over several generations. Middens accumulated directly adjacent to these building clusters and within abandoned buildings, forming large areas of midden material, including ash and charcoal, animal bone, construction debris and organic waste (Shillito & Matthews 2013). Human coprolites and animal dung were occasionally found preserved in these open middens (Shillito *et al.* 2011a & b). In contrast, building interiors were kept remarkably clean, even at the microscale, with frequent sweeping out and re-plastering of floors and walls on a seasonal basis (Matthews 2005; Matthews *et al.* 2014).

<FIGURE 1, 13.5cm greyscale>

While there has been research on the human skeletal remains from Çatalhöyük to investigate disease and diet in the population (e.g. Hillson *et al.* 2013; Larsen *et al.* 2015; Pearson *et al.* 2015), intestinal disease is not easily assessed from study of the skeleton. Instead, faecal material preserved in an identifiable archaeological context is required. In societies pre-dating the invention of the toilet in the fourth millennium BC (McMahon 2015), the best targets for analysis are coprolites (preserved pieces of human faeces) and samples of sediment from the pelvic area of burials, where the intestines would have been located during life (Reinhard *et al.* 1986; Mitchell 2017a). Analysis of coprolites and pelvic sediment can provide evidence for the species of intestinal parasites that infected individuals in a population and, if sample sizes are large enough, it may also provide an indication of how widespread infection was, and whether some people were more heavily infected than others.

Materials

The four coprolites analysed here were recovered during excavation of space 329, Level South P (Figure 2), dating to *c.* 6410–6150 BC (from radiocarbon dates interpreted with Bayesian modelling). This corresponds to late or 'post-peak' occupation of the site, as defined by Hillson *et al.* (2013). Space 329 comprises a series of laminated, midden-like deposits interspersed with charred spots indicating *in situ* burning activity (Reagan & Taylor

2009). Each coprolite was mineralised (Figure 3), being either hard and solid, or having a crumbly texture with some hard portions.

<FIGURE 2, 13.5cm greyscale>

<FIGURE 3, 6.5cm colour, place to left of text and wrap around>

Six human burials were also analysed (for an example, see Figure 4). These individuals were all interred beneath the platforms found within houses (see Figure 2). The burials date from 7100–6300 BC; further details are provided in Table 1. Sediment samples were taken from the area anterior to the sacrum where the intestines would be positioned, and control samples were obtained from within the cranial vault and foot area of each inhumation, where the eggs of intestinal parasites should not be found during life (Reinhard *et al.* 1986; Mitchell 2017a).

<FIGURE 4, 13.5cm colour>

<TABLE 1>

Methods

One-gram samples of each dried coprolite were weighed, fragmented using a pestle and mortar and disaggregated with 0.5 per cent trisodium phosphate for 72 hours. The mineralised coprolite samples also required a small quantity of dilute hydrochloric acid (10 per cent HCl) to disaggregate the samples. The HCl was added drop by drop to break down calcium carbonates until the gentle bubbling ceased. The pelvic soil samples did not require the use of a pestle and mortar or HCl, and disaggregated completely with 0.5 per cent trisodium phosphate. Once the material was in suspension, each sample was passed through a stack of microsieves with mesh sizes of 300 μ m, 160 μ m and 20 μ m. As intestinal parasite eggs found in this region of the world are typically between 20 and 150 μ m in size, they are trapped on the 20 μ m mesh, while larger and smaller sized soil particles are removed. Sediment trapped on the 20 μ m mesh was washed free with ultrapure water and centrifuged to concentrate it. The supernatant was then removed, glycerol added and the sample viewed using digital light microscopy at 400 \times magnification (see Anastasiou & Mitchell 2013; Dufour & Le Bailly 2013).

Coprolites found to be positive for parasite eggs underwent faecal lipid analysis targeting both sterols and bile acids to determine whether they were of human or animal origin. For faecal lipid analysis, samples were prepared according to methods described in Bull *et al.* (2002). In summary, two internal standards (hyocholic acid and preg-5-en-3 β -ol, 0.1 mg mL⁻¹ solution) were added to 1g of sieved sample. Samples were processed by microwave-assisted solvent and then saponified. The saponified total lipid extracted was then separated into

neutral and acid fractions using aminopropyl SPE columns. The neutral fraction was separated using a silica gel column into less polar and sterol fractions. Sterol fractions and bile acids were derivatised by the addition of BSTFA/TMCS (99:1 v/v). Excess derivatisation agent was evaporated under a gentle stream of nitrogen and the sample redissolved in ethyl acetate prior to analysis by gas chromatography (GC) and gas chromatography mass spectrometry (GC/MS). The carboxylic fraction was methylated using TMS-DAM in toluene/methanol (4:1 v/v). The methylated acids were further separated using silica gel columns into mono- and dicarboxylic fatty acid methyl esters/diesters, and hydroxylated carboxylic acid methyl esters, the latter containing the bile acids. GC/MS peak assignments were made by comparison with known mass spectra (NIST08 and laboratory reference library) and, where possible, authentic standards. Species identifications were made through calculation of sterol ratios in conjunction with bile acid profiles.

Results

Two coprolites, 17384 s.3.1 and 17384 s.4.1, tested positive for whipworm eggs (*Trichuris* sp.) (Figure 5). A total of 12 eggs were recovered: four from s.3.1, and eight from s.4.1. The eggs were identified by their lemon-shaped form, brown colour, location of apical plugs—although the plugs themselves were lost due to decomposition—and egg dimensions (Table 2). No parasite eggs were identified in the analysed pelvic soil samples.

<FIGURE 5, 6.5cm colour, place to left of text and wrap around>

<TABLE 2>

The sterol profiles for both samples show a clear omnivore signal, with a high proportion of coprostanol, smaller proportions of cholesterol and a range of phytosterols (see Figures 6–7). The ratios are presented in Table 3. The bile acid profiles for both samples were dominated by deoxycholic acid, with smaller proportions of lithocholic acids—a signal that indicates a human origin. Other potential omnivores, such as dog and pig, are excluded, as dogs produce negligible quantities of coprostanol with much higher proportions of cholesterol; the bile acid profile for pigs contains a significant proportion of hyodeoxycholic acid, which is not present in either of the samples analysed.

<FIGURE 6, 13.5cm greyscale>

<FIGURE 7, 13.5cm greyscale>

<TABLE 3>

Discussion

This is the first evidence for intestinal parasite infection at a Neolithic settlement in the mainland Near East. Two of the four coprolites analysed tested positive for parasite eggs, while the pelvic soil samples from the six burials were all negative. Sterol and bile acid analysis was undertaken to identify whether humans or animals deposited the coprolites. Faecal biomarker analysis to determine which species produced a particular coprolite has become a well-established method in archaeology (see Shillito *et al.* 2011b; Shillito *et al.* 2013; Prost *et al.* 2017). Identification of coprolites gives important information about the host of the parasite and enables researchers to identify taxonomically parasite species that infect different hosts, but whose eggs cannot be distinguished morphologically by microscopic investigation. We did not analyse the pelvic soil samples for faecal biomarkers, as human origin would be clear from skeletal morphology. Sterol profiles of coprolite samples are indicative of carnivore, herbivore or omnivore diet, while bile acids can distinguish between omnivores, such as pigs and humans (Bull *et al.* 1999).

In the coprolites analysed here, the high levels of coprostanol, but low levels of cholesterol and phytosterols indicate an omnivore, rather than a carnivore or herbivore signal. The bile acid analysis shows a dominance of deoxycholic acid with smaller amounts of lithocholic acids. This indicates that the faeces were of human origin and not from other omnivores, such as pigs, which might defecate on refuse areas as they scavenge for scraps.

The presence of whipworm eggs in these two coprolites indicates that the faeces forming the coprolites came from humans who were infected with this parasite. It is not possible to determine whether each coprolite came from different individuals or both from the same individual, but from different defecation events. Both human whipworm (*T. trichiura*) and pig whipworm (*T. suis*) produce eggs of similar appearance on microscopic investigation. Although pig whipworm eggs do tend to be a little larger than human whipworm, there is some overlap in their size ranges (Beer 1976; Mehlhorn 2016). Analysis of sterols and bile acids, however, increase the probability that these eggs are from the human whipworm (*T. trichiura*).

Whipworm is a parasite that is closely linked to lifestyle and environmental conditions such as sanitation, as it is spread by the faecal contamination of food and water. Human whipworm is thought to have evolved in human ancestors in Africa and spread with early human migrations (Mitchell 2013); it is a species therefore expected to be present in early populations. Adult worms are 30–50mm in length and they live on the intestinal lining of the large bowel (Garcia 2016: 311). The lifecycle involves an infected person defecating, the whipworm eggs then develop in a damp, shady environment for 1–2 weeks, by which time

they mature and reach their infective form. If the eggs are then swallowed by another person with their food or water, the eggs hatch in the intestines, develop into adult worms, mate and lay eggs, which mix in with the faeces. Whipworms typically live for up to five years (Weller & Nutman 2014).

The egg concentration recovered from these coprolites was low, at 4–8 eggs/g. As egg concentrations are often lower in pelvic soil than coprolites, the absence of eggs in the soil samples may be false negatives. A female whipworm typically produces around 5000 eggs each day (Weller & Nutman 2014). Low egg counts therefore probably indicate that many of the eggs originally present have not survived the last 8000 years. Moreover, it is possible that eggs from other species were present in lower concentrations and are therefore not represented in our samples. Parasite eggs in the soil can be damaged and destroyed by the action of fungi and insects, or washed away by water flowing through the sample over the centuries (Morrow *et al.* 2016). Sample preparation can also damage parasite eggs. The use of concentrated hydrochloric acid (36 per cent) during sample processing, for example, has also been shown to damage the eggs of several species of parasite, such as *Capillaria hepatica*, although this procedure has little effect on the eggs of most other parasite species (Dufour & Le Bailly 2013). While the dilute acid (10 per cent HCl) used here was necessary to disaggregate the mineralised coprolites, it is possible that it may have reduced the number of eggs in the sample.

If the people who deposited these coprolites genuinely had a low number of worms in their intestines, they may have been asymptomatic, and their health may not have been impaired by the infection. For individuals who are infected by many worms, however, research on modern patients shows that whipworm infection can lead to abdominal pain, anaemia, diarrhoea, stunted growth and reduced intelligence in children (Stephenson *et al.* 2000; Bethony *et al.* 2006; Jourdan *et al.* 2018). Growth has been studied in juvenile skeletons from Çatalhöyük. Comparison of stature and body mass estimates with those from skeletal collections of well-nourished individuals indicates that health and nutrition were adequate to maintain similar rates of growth in children at Çatalhöyük (Hillson *et al.* 2013). Skeletal indicators of anaemia, such as *cribra orbitalia*, have been recorded in the human remains, especially in infants (Molleson *et al.* 2005). It is not easy to differentiate whether this anaemia might be related to whipworm infection, to a diet low in iron or folate, or to the presence of other infections, such as malaria.

Past parasite analysis at other Neolithic sites in the Mediterranean region has shown a mixed picture of intestinal infection. There tend to be zoonotic species that spread to humans from

animals, and geohelminths, which are spread from one human to another by the contamination of food or water with human faeces. At Çatalhöyük, we have only found evidence for the geohelminth whipworm. Parasite species in Mediterranean populations during the Bronze Age, Iron Age, Roman and Medieval periods, however, were dominated by whipworm and roundworm (geohelminths), with the parasite species requiring animals to complete their lifecycles becoming increasingly rare (Mitchell 2015b, 2017b; Williams *et al.* 2017; Anastasiou *et al.* 2018; Ledger *et al.* 2018). This might be explained by a change in lifestyle from the Neolithic mixed subsistence pattern of farming coupled with the hunting of wild animals and the gathering of wild produce, to later subsistence that was heavily reliant upon farming cereals, eating fruit and herding domesticated animals, such as sheep and goats (Petroutsa & Manolis 2010; Pokutta 2017). We should also consider the possibility that preservation conditions in the Mediterranean region may contribute to the apparent reduction of parasite species diversity over time. We would, however, expect that species that have equally robust eggs to whipworm—*Taenia* tapeworms, for example—could also survive in this environment.

Çatalhöyük is particularly interesting, as it represents one of the best-preserved, large Neolithic settlement agglomerations in the Near East. Excavation has demonstrated the population's reliance upon cereal crops and animal herding for subsistence. While plant remains from the site do include legumes and fruit and nuts, the vast majority of domesticated plant remains were of wheat and barley (Ryan 2008; Bogaard *et al.* 2013, 2017). Domestic sheep and goats were eaten regularly and were overwhelmingly the most common animal species found at the site, whereas cattle were eaten at feasts (Bogaard *et al.* 2013; Russell *et al.* 2013). While there is some evidence for the consumption of wild deer, wild horse, birds, wild pigs and small freshwater fish, most of the meat consumed was from domesticated sheep and goats (Russell *et al.* 2013; Van Neer *et al.* 2013).

Diet at Çatalhöyük appears to resemble more closely that of Mediterranean Bronze Age and Iron Age populations than other Neolithic sites that have previously been studied for parasites in Cyprus and Spain (Harter-Lailheugue *et al.* 2005; Maicher *et al.* 2017). The research presented here suggests that the parasite species present at Çatalhöyük were also unlike those present at the other aforementioned Neolithic sites in the Mediterranean. In a pattern similar to that observed in later periods in the Mediterranean, geohelminths were dominant at Neolithic Çatalhöyük, while zoonotic parasites were not found in the analysed samples. Crowded living conditions, with middens containing human excrement located directly adjacent to houses, probably played a role in the transmission of whipworm, as it is spread by

the faecal-oral route; a move away from reliance on wild animals may have decreased the presence of zoonotic species. We do need to consider the limited size of our sample; it is possible that other parasite species might be identified if future work recovers more coprolites from elsewhere at the site. From the evidence acquired so far, however, it appears that the lifestyle and diet of the inhabitants of Çatalhöyük resulted in fewer species of intestinal parasite in the population than were present at other Neolithic sites in the Mediterranean region.

Conclusion

Through analysis of both coprolites and pelvic soil from burials at Çatalhöyük, this study has investigated intestinal parasitic infection in the inhabitants of the large Neolithic settlement over 8000 years ago. The results demonstrate that a proportion of the population was infected with whipworm. While the sample size is small, we have found fewer species of parasite at this site than is the case for studies of other Neolithic sites in Cyprus and Spain. The pattern at Çatalhöyük is more representative of what would be expected at Bronze and Iron Age sites in the Mediterranean. This would suggest that the organised nature of Çatalhöyük—its housing, infrastructure, socio-cultural practices and subsistence strategies—may have reduced the diversity of parasitic infection among the inhabitants of the site, a phenomenon more normally associated with much later time periods.

Acknowledgements

The parasite analysis was supported by a doctoral award from the Social Sciences and Humanities Research Council of Canada (752-2016-2085) and by a Tidmarsh Cambridge Scholarship from the Cambridge Commonwealth, European and International Trust and Trinity Hall College (to M.L.L.). This study received financial support from the French State under the ‘Investments for the Future’ programme, ‘Initiative d’Excellence’, Université Bordeaux (ANR-10-IDEX-03-02) to C.J.K.).

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Figure captions

Figure 1. Map showing the location of Çatalhöyük within Turkey (figure from the Çatalhöyük Research Project).

Figure 2. Plan showing the location of the coprolites and burials analysed (figure by Camilla Mazzucato).

Figure 3. Coprolite 15702 s.21.1, with dimensions 41mm in length and 23mm in width (figure by Evilena Anastasiou).

Figure 4. Skeleton 30928, a possible female, aged 35–50 years, dating from 6700–6500 BC (figure from the Çatalhöyük Research Project).

Figure 5. Whipworm egg from coprolite 17384s 4.1. Dimensions $23.1 \times 50.2\mu\text{m}$ (figure by Evilena Anastasiou).

Figure 6. Bile acid profile for 17384 s3.1 (figure by Lisa-Marie Shillito & Ian Bull).

Figure 7. Sterol profiles for 17384 s3.1 (figure by Lisa-Marie Shillito & Ian Bull).

Table 1. Details of the burials from which pelvic soil samples were taken, including age, sex, location and date.

Sample number	Burial ID	Age	Sex	Location	Hodder level	Date
30348.s3	Burial F.7508 skeleton 30351	20–35 years	Possible female	South area, building 43	South L	7100–6700 BC
	Burial F.3484 skeleton 30927	9±3 months	Unknown	South area, building 89	South N	6700–6500 BC
30929.s8	Burial F.3484 skeleton 30928	35–50 years	Possible female	South area, building 89	South N	6700–6500 BC
19435.s3	Burial F.3629 skeleton 8598	20–35 years	Male	North area, building 114	North G	6700–6500 BC
22027.s5	Burial F.7333 skeleton 22026	12±2.5 years	Unknown	North area, building 77	North G	6700–6500 BC

20450.s14	Burial F.3630 skeleton 19460	11±2.5 years	Unknown	North area, building 129	North H	6500–6300 BC
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Table 2. Details of the whipworm eggs found in a 1g subsample from each coprolite.

Coprolite ID	No. of eggs	Length (µm)			Width (µm)		
		Mean	SD	Range	Mean	SD	Range
17384s.3.1	4	46.9	1.4	46.2– 49.6	23.6	1.6	22.7–25.1
17384s.4.1	8	47.7	1.5	46.2– 50.5	22.7	1.4	20.1–25.3

Table 3. Sterol profiles for the two coprolites testing positive for parasite eggs.

Sample	Location	Hodder level	Mellaart level	Ratio 1	Ratio 2	Bile acids
CH 17384 s4.1	South area, space 329, unit 17384	South P	Uncertain V	0.95	1.77	Dominated by deoxycholic acid with small amounts of lithocholic and isolithocholic acid.
CH 17384 s3.1	South area, space 329, unit 17384	South P	Uncertain V	0.95	1.47	Dominated by deoxycholic acid with small amounts of lithocholic and isolithocholic

acid. Trace quantities of
chenodeoxycholic acid.
