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Case study

Implications of the prevalence of *Ascaris* sp. in the funerary context of a Late Antique population (5th-7th c.) in Granada (Spain)

Ramón López-Gijón [a,*], Edgard Camarós [b], Ángel Rubio-Salvador [a], Salvatore Duras [a], Miguel C. Botella-López [a], Inmaculada Alemán-Aguilera [a], Ángel Rodríguez-Aguilera [c], Macarena Bustamante-Álvarez [d], Lydia P. Sánchez-Barba [a], Benjamin Dufour [e], Matthieu Le Bailly [e]

[a] Laboratorio de Antropología, Facultad de Medicina, Universidad de Granada, Av. de la Investigación 11, 18071 Granada, Spain
[b] Departamento de Historia (Sección de Prehistoria), Universidad de Santiago de Compostela, Praza da Universidade, 15703 Santiago de Compostela, Spain
[c] Gespad al-Andalus SLU, Era del Cura 20, 18170 Alfacar, Spain
[d] Departamento de Prehistoria y Arqueología, Facultad de Filosofía y Letras, Campus de Cartuja s/n, 18016 Granada, Spain
[e] CNRS UMR 6249 Chrono-environment, University of Franche-Comte, 16 route de Gray, 25030 Besançon Cedex, France

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ABSTRACT

Objective: To evaluate the prevalence of gastro-intestinal parasites in human remains from Late Antiquity (5th – 7th c.) Granada (Spain).
Materials: The study included pelvic and cranial control samples from 17 skeletons from the archaeological sites of Los Mondragones (n = 13) and Rafael Guillén (n = 4).
Methods: In the paleoparasitological study, soil samples from pelvic area and cranium were analyzed using the rehydration, homogenization, and micro-sieving method and visualization under brightfield microscopy.
Results: Ascaris sp. eggs were detected in pelvic samples from seven individuals.
Conclusions: These findings may indicate that this parasite was endemic. Its detection frequency is one of the highest reported at group level in an osteological series from Late Antiquity.
Significance: The prevalence of *Ascaris* sp. associated with skeletal remains has implications for assessing the lifestyle and health of populations in southern Spain during the Late Antique period.
Limitations: The number of individuals is small and taphonomic processes could have limited paleoparasitological findings
Suggestions for further research: Future interdisciplinary studies of this type are warranted in larger osteological series to improve knowledge of parasitosis in the past.

1. Introduction

Paleoparasitological research can contribute to our knowledge of cultural developments and environmental stress in past populations (Bouchet et al., 2003a; Le Bailly et al., 2021). Deeper understanding can be achieved by interdisciplinary studies involving researchers from different scientific fields (Buikstra et al., 2022). In this way, the combination of paleoparasitological, anthropological, and archaeological data can provide further insight into the sanitary conditions, health status, and lifeways of ancient people and into their interactions with each other and their environment (Reinhard et al., 2013; Araújo et al., 2015).

Studies of parasites from European archaeological sites have described a predominance of geohelminths in ancient human populations, especially roundworm (*Ascaris* sp.) and whipworm (*Trichuris* sp.) (Anastasiou, 2015; Gaeta and Fornaciari, 2022). They have mainly been detected in fecal materials preserved in latrines, cesspits, and sewer drains, among other archaeological structures (Mitchell, 2016).

Despite the value of relating parasitosis to individuals and their biological profile, there has been less research on its presence associated with skeletal remains or its prevalence in specific populations, with a few exceptions (e.g., Anastasiou et al., 2018; Roche et al., 2019; Ledger et al., 2021). In general, save a small number of publications (e.g., Cunha et al., 2017; López-Gijón et al., 2022), studies that combine

* Corresponding author.
E-mail address: ramonlopez131094@correo.ugr.es (R. López-Gijón).

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parasitism with biological profile data have been merely anecdotal, with reduced sample size as the main limitation alongside the infrequent involvement in excavations of specialists in the collection of parasite samples and negative taphonomic effects on the preservation of parasitic evidence (Morrow et al., 2016; Camacho et al., 2020; Ramírez et al., 2022). The substantial and irreparable loss of this evidence often limits this type of study to larger bone collections excavated before the standardization of paleoparasitological techniques.

Even less information on parasitosis has been obtained from human remains in Europe in the Late Antiquity (4th-8th c.) (Cameron, 2015; Dufour et al., 2016; Roche et al., 2019; 2021; Ledger et al., 2021) than it has been in the earlier Roman (Dufour, 2015; Ledger et al., 2020; Mitchell, 2023a) and later Medieval (Mitchell, 2023b) periods, with only one case reported from this time in the Iberian Peninsula (López-Gijón et al., 2023). The objectives of this study were to report on findings from the Late Antique necropolis of Los Mondragones and Plaza Rafael Guillén in Granada, Spain, and to explore their implications for understanding parasitosis, especially by intestinal helminth, in an ancient population.

2. Materials and methods

2.1. The archaeological sites

The studied individuals come from two archaeological sites, Los Mondragones and Plaza Rafael Guillén, in settlements on the outskirts of ancient *Municipium Florentinum Iliberritanum* (Granada, Spain) (see Fig. 1).

Los Mondragones is in an agricultural area to the north of the city on the right bank of the river Beiro. The villa was constructed in the 1st century AD and possesses a *torcularium* and *calcatorium* as part of its productive base, reflecting its importance. It underwent complex development from the second half of the 4th century CE up to the 7th century CE, acquiring certain urban characteristics (Rodríguez-Aguilera et al., 2014, 2020). A bathhouse with residential facilities was reorganized during Late Antiquity but retained architectural features from the Roman period, including rooms for thermal circuits and a space for a possible palestra. Burial spaces were found near the most important buildings in the settlement, including a church from the 6th century. A total of 134 individuals were recovered, 121 from the initial excavation (Fernández-Martínez et al., 2020; Fernández-Martínez, 2020) and 13 from a later excavation. The 13 individuals underwent paleoparasitological study. The finding of two epigraphs during the later excavation, one in stone and one in bronze (Ventura Villanueva et al., 2023), suggests that the Los Mondragones site might have formed part of the estates of *Mummius Modestus*, a member of the urban aristocracy of *Florentia Iliberritana*. He was an ancestor of *Mummii Nigri Velerii Vegeti*, which was one of the most important Senatorial families in Roman Granada and responsible for endowing the *Municipium Iliberritanum* with the legacy of *Mummius Modestus* (Ventura Villanueva et al. 2023).

The second site, the Roman villa of Plaza Rafael Guillén, is located to the south of the city, 4 kilometers from the archaeological site of Mondragones. It was also founded in the 1st-2nd centuries CE and underwent a similar development in the mid-4th century CE (Fresneda et al., 1991). Its later existence is only indicated by four tombs from the 5th to 7th centuries CE, comprising three individual tombs and a fourth double tomb with associated grave goods (small jar and some personal bronze adornments). This villa is also likely to have formed part of the estates of an aristocratic family in Roman times, but no epigraphic element has been found to allow its identification.

The main difference between the two settlements is that the villa of Plaza Rafael Guillén was occupied in an occasional and sporadic

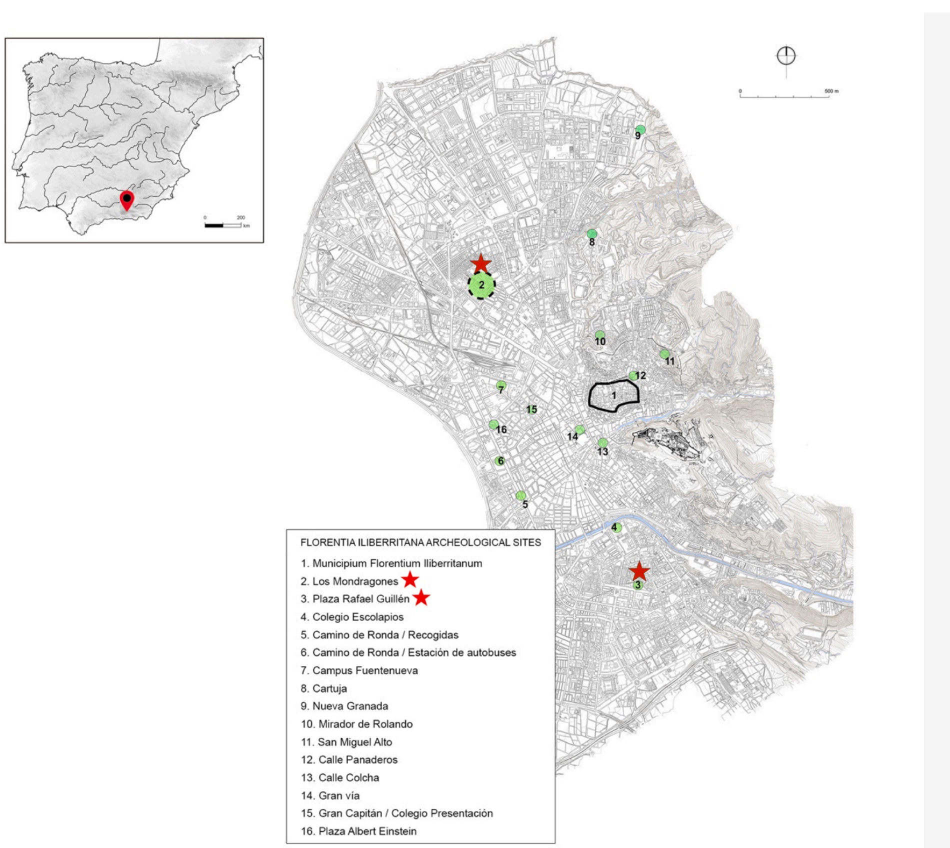

Fig. 1. Map showing the location of the current city of Granada within which both sites are found and the plan showing the location of the *Florentia Iliberritana* archaeological sites in the modern city.

manner, whereas Los Mondragones was in continuous occupation and even became revitalized at a time when the ancient Roman city was in decline (Rodríguez-Aguilera et al., 2020).

2.2. Methodology

The skeletal remains from these sites are in the custody of the Anthropology Laboratory of the University of Granada. The Mondragones collection comprises 13 individuals (5 sub-adults and 8 adults) sampled during archaeological excavations from 2018 to 2020 when, for the first time, sediment samples were systematically collected in situ during excavation of the skeletal remains. The same approach was followed in excavating the four tombs at the Plaza Rafael Guillen site (2020 excavation), which contained the four subadults described in the present study (sediment samples were not collected for paleoparasitological study from a fifth individual in the double tomb). All of these tombs and their contents were intact, and all skeletal remains were in an excellent state of preservation. In addition, all individuals were in primary burials (see Fig. 2a), allowing sampling to be conducted in full accordance with standard protocols (Reinhard et al., 1992; Le Bailly et al., 2021).

The sex of adults was estimated using standard biological anthropology techniques (Buikstra and Ubelaker, 1994), and their age-at-death was estimated from the ectocranial suture closure (Meindl and Lovejoy, 1985) and morphology of the auricular surface (Lovejoy et al., 1985) and pubic symphysis (Brooks and Suchey, 1990). Estimation of the age-at-death of subadults was based on dental eruption (Alqahtani et al., 2010), tooth length (Irurita Olivares et al., 2014), epiphyseal fusion (Cardoso, 2008a; 2008b), and long bone diaphysis length (Cardoso et al., 2014; Fazékas and Kósa, 1978).

Sampling of intestinal parasites was carried out in situ, extracting sediment from the pelvic area for the test sample and from the cranium for the corresponding control sample, following Le Bailly et al. (2021). The rehydration, homogenization, and micro sieving (RHM) technique, perfected at the University of Franche-Comté (Dufour and Le Bailly, 2013), was utilized for the paleoparasitological study. Briefly, 1 g of each sediment sample was hydrated for seven days in an aqueous solution containing 50% 0.5% trisodium phosphate/50% 5% glycerol along with drops of formaldehyde to prevent fungal growth. After rehydration, the sample was crushed in a porcelain mortar, and the resulting material was immersed in an ultrasound bath for one minute. Next, the material was strained through a micro-sieve column of 315 μm, 160 μm, 50 μm and 25 μm meshes under a constant flux of water. Contents of the 50 μm and 25 μm meshes were then collected, corresponding to the sizes of parasite eggs found in ancient material (Bouchet et al., 2003b).

Bright-field transmitted light optical microscopy was used to visualize 20 preparations per sample under 100x, 400x, and 630x magnification, capturing photographs at 630x magnification.

3. Results

A total of 680 slides were analyzed from the 34 soil samples gathered at the two sites, 17 from pelvic areas and 17 from crania (controls). No eggs were found in any control sample. Among the pelvic area samples, no eggs were detected in 10 and *Ascaris* sp. eggs (n = 30) in 7. These eggs were found in 6 (46%) of the 13 individuals from Los Mondragones, and 1 (25%) of the 4 from the Rafael Guillén site (Table 1).

The morphology and dimensions (55.8–66.8 μm long, 41.9–50.9 wide) of the eggs in the seven positive samples are compatible with *Ascaris* sp., which have a characteristic mamillated shell and ovoid/elliptic shape (Fig. 2b). Some of the eggs were decorticated (Fig. 2c), and eggs of this species are frequently found without their mamillated coat in paleoparasitological studies.

The five subadults from the Los Mondragones site ranged from perinatal to infant ages. The only positive sample was from an Infant (Ind. 137) with an estimated age of 1–2 years. From the Plaza Rafael Guillén series, *Ascaris* sp. was only detected in one individual (Ind. 2025), an adolescent aged around 14–16 years old (Table 1).

A higher prevalence of parasites was observed in the eight adults (62%), who were all from Los Mondragones, with eggs of *Ascaris sp.* found in the two males and associated with three of the six females. By age, the males were both young adults, while two of the infected females were old adults (Table 1).

4. Discussion

The findings in the seven infected individuals from the two sites are compatible with *Ascaris sp.*, since they are between 45 and 75 μm in length and 35–50 μm in width, which is within the size range of *Ascaris sp.* eggs, (Thienpont et al., 1986; Roberts et al., 2008). However, it is

Fig. 2. a) Individual found at Los Mondragones; b) Egg of *Ascaris* sp. (62.5 × 49.9 μm); c) Decorticated egg of *Ascaris* sp. (66.8 × 45.4 μm).

Table 1
Details of parasite eggs and anthropological findings in human remains from Los Mondragones and Rafael Guillén.

Tomb	Number of *Ascaris* sp. eggs	Sex	Age class and range of age	Chronology
Mondragones site				
137	2	-	Infant (1–2 years)	**V-VI**
232	-	♀	Young adult (21–35 years)	**V-VI**
234	5	♀	Old Adult (50+ years)	**V-VI**
235	-	-	Infant (3–7 months)	**V-VI**
239	3	♂	Young Adult (21–35 years)	**V-VI**
240B	-	-	Infant (1–2 years)	**V-VI**
291	12	♀	Old Adult (50+ years)	**V-VI**
314A	-	♀	Young Adult (21–35 years)	**V-VI**
356	2	♂	Young Adult (21–35 years)	**V-VI**
388	-	-	Infant (6–10 months)	**V-VI**
398	3	♀	Old Adult (50+years)	**V-VI**
1255	-	♀	Old Adult (50+ years)	**V-VI**
1258	-	-	Perinatal (circa 40 weeks)	**V-VI**
Plaza Rafael Guillén site				
2025	3	-	Adolescent (14–16 years)	**V**
2023	-	-	Children (9–11 years)	**V**
2033b	-	-	Children (7–9 years)	**VI-VII**
11045	-	-	Adolescent 17–20 years	**VI-VII**

very difficult to differentiate morphologically or genetically (Liu et al., 2012; Leles et al., 2012) between *A. lumbricoides*, observed in humans, and *A. suum*, which is found in pigs, but can occasionally infect humans (Nejsum et al., 2012; Vlaminck et al., 2014). Given their detection in soil samples from skeletal human remains, it can be assumed that the detected eggs are *A. lumbricoides* (Šebela et al., 1990; Le Bailly et al., 2014; Rácz et al., 2015; Wang et al., 2022), although reports of domesticated pigs at Los Mondragones (Fernández-Martínez et al., 2020; Fernández-Martínez, 2020) mean that coinfection cannot be ruled out.

Only 30 eggs were gathered from the soil samples, and they all belonged to roundworm, likely attributable to the poor preservation of parasites in archaeological materials. The postmortem loss of parasite eggs can be attributed to taphonomic factors (Ramírez et al., 2022), including the environmental effects of water percolation (Camacho et al., 2016) and of soil pH and composition (Morrow et al., 2016). Both archaeological sites under investigation are in the southeast of the Iberian Peninsula, characterized by a semi-arid climate, which is less favorable for parasite preservation (Bouchet et al., 2003a; Mitchell et al., 2022) in comparison to humid environments, such as that in the north of the Peninsula (Maicher et al., 2017; López-Gijón et al., 2022). These problems may account for the absence of *Trichuris sp*, which are usually associated with the presence of *Ascaris sp.* but are more susceptible to taphonomic processes (Rousset et al., 1996; Ledger et al., 2021; Roche et al., 2021; Wang et al., 2022). The finding of *Ascaris sp.* but not *Trichuris sp.* was previously reported at a site with similar chronology and geography (López-Gijón et al., 2022) and in materials with later chronologies from nearby areas, as in the study of Medieval Islamic latrines in Cordoba (Knorr et al., 2019). The shells of *Ascaris sp.* are particularly resistant, having an outer protein layer, a middle chitinous layer, and an inner lipoid layer (Wharton, 1980), favoring their preservation over time. In addition, the females produce around 200,000 eggs daily (Roberts et al., (2008)), further explaining why it is one of the most frequently detected parasites in European archaeological studies (Bouchet et al., 2003b).

The observation of parasites in osteological series can yield crucial information about the biological profile of parasitized individuals, potentially offering valuable insights into lifeways of populations and sources of parasitic infection. The prevalence of parasites was 40% in individuals from the two sites. The prevalence of 46% at Los Mondragones (6/13 cases) is one of the highest reported for ascariasis in Europe except for the Uffizi Gallery, where the application of genetic analysis increased the number of parasitized individuals detected (Roche et al., 2021) (Table 2). The prevalence was lower among subadults than among adults from these sites, although the subadults from Los Mondragones included three infants aged under one year, and current clinical data indicate that parasite infections below the age of six months are rare (Scott, 2008; Jourdan et al., 2018), as are congenital parasitic infections (MacLeod (1987)).

Various pathways of infection may explain the present findings, including the consumption of contaminated food or water or contact with a contaminated environment (Bethony et al., 2006). The youngest positive individual (1–2 years-old) may have been infected by the intake of supplementary food (Bowman, 2021) or by the typical interaction of young infants with their surrounds, including geophagy, which could expose them to parasite eggs in the soil (López-Gijón et al., 2023).

Isotopic studies at this site have revealed that individuals consumed an adult diet from the age of 4 years (Fernández-Martínez et al., 2020). Hence, Ind. 2025, aged 14–16 years, might have become parasitized in the same way as the adults from the consumption of contaminated food products. This individual and the young adult males may also have participated in agricultural activities that involve potential sources of exposure such as the human fecal material that served as fertilizer (Mitchell, 2017) or the wastewater used for irrigation (Amahmid et al., 1999; Landa-Cansigno et al., 2013).

Unlike current populations, in which roundworm is more frequent in children than in other age groups, (Crompton, 1988; De Silva et al., 2003; Mascarini-Serra, 2011; Holland et al., 2022), more positive samples were found in the adults than in the subadults in the present study. This may be attributable to the aforementioned low propensity of young infants to roundworm infection (three individuals were aged $<$1 year and two aged $<$2 years in this study), the fact that infection can only be detected in those who died while infected, and the scant number of children at these sites (only 2 individuals). The small number of individuals is a limitation of this study, but offers a first analysis of parasitosis in this society.

In general, the present findings might indicate a domestic or peridomestic environment that is contaminated by the substrate itself, the presence of contaminated water (Asaolu et al., 2002), or food, or the poor management of fecal material (Esrey et al., 1991; Fung and Cairncross, 2009; Bowman, 2021). Regardless, the detection of parasites in this type of material should be considered from a socio-economic and cultural perspective (Slepchenko et al., 2020). In the present case, the Roman city of *Florentia Iliberritana* was strongly associated with agricultural activity, especially the "Mediterranean triad" of grapes, wheat, and olives, exemplified by the oil press found in Mondragones (Punzón, 2014; Fernández-Martínez et al., 2020; Rodríguez-Aguilera et al., 2014).

There is historic evidence of the utilization of human feces as fertilizer, which may contribute to the spread of roundworm (Jones, 2016).

Table 2
Ascaris sp. found in Late Antique period individuals.

Archaeological settlement	Chronology	Prevalence of *Ascaris* sp. in human remains	Reference
Uffizi Gallery (Italy)	4th – 5th C.	5/18 (28%)3/5 (60%)	Roche et al. (2019, 2021)
Mondragones (Spain)	5th - 6th C.	6/13 (46%)	Present article
Vacone (Italy)	3th – 7th C.	1/3 (33%)	Ledger et al. (2021)
Plaza Rafael Guillén (Spain)	5th - 7th C.	1/4 (25%)	Present article
Selvicciola (Italy)	4th – 8th C.	3/15 (20%)	Ledger et al. (2021)

More specifically, paleoparasitological studies of material from Roman and Late Antique periods have pointed to the role of human feces in farming (Mitchell, 2017; Ledger et al., 2021). Hence, this practice may be linked to the present findings, given the importance of agriculture in the area during Late Antiquity, when the city was called Eliberri and became an important religious and political center responsible for multiple coin mintings (Punzón, 2014).

The resulting exposure to parasitosis would affect not only individuals directly engaged in agricultural activities but also those handling and consuming contaminated food products, including subadults and old adults (Klapek and Borecka, 2012; Amahmid et al. 2022). The severity of health effects depends on the number of roundworms involved in the infection, with some infected individuals being asymptomatic but others experiencing diarrhea, abdominal cramps, anemia, stunted childhood growth, and even intestinal obstruction (Jourdan et al. 2018).

5. Conclusion

This study of 17 skeletonized individuals from two sites in Granada, Spain (Los Mondragones and Rafael Guillén) contributes the first evidence of the presence of *Ascaris* sp. in Late Antiquity populations in the Iberian Peninsula. The prevalence at both sites is in the high mid-range of reports on similar material from the same period in Europe. These findings suggest that infection with this parasite was endemic in the Southeastern Iberian Peninsula. This study contributes data on the prevalence of this type of parasitosis in a group comprising individuals of different ages and both sexes. The utilization of human fecal material as fertilizer, the irrigation of crops with sewage, the intake of contaminated food, and hygiene deficiencies may be responsible for a high prevalence of parasitosis in farming communities. The role played by these parasites in the health and disease of past populations is likely to be underestimated due to the scarcity of population-based analyses, which can provide novel information on their hygienic-sanitary conditions.

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Declaration of Competing Interest

The authors state that they do not have any conflict of interest to declare.

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