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Parasite eggs in 16th-18th century cesspits from Granada (Spain)

Ramón López-Gijón^{a,*}, Sylvia Jiménez-Brobeil^a, Rosa Maroto-Benavides^a, Salvatore Duras^a,
Amjad Suliman^b, Pablo L. Fernández Romero^b, Miguel C. Botella-López^a,
Francisco Sánchez-Montes^c, Piers D. Mitchell^d

^a Laboratory of Anthropology, Faculty of Medicine, University of Granada, Av. de la Investigación 11, 18071 Granada, Spain^b Independent Archaeologist, Spain^c Department of Modern and American History, Faculty of Philosophy and Letters, University of Granada, Campus Universitario de Cartuja, 18071 Granada, Spain^d Department of Archaeology, University of Cambridge, The Henry Wellcome Building, Fitzwilliam Street, Cambridge CB2 1QH, UK

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ABSTRACT

The study of parasites from archaeological materials can yield information on socioeconomic conditions, as well as hygiene and waste management. The investigation of contemporaneous texts offers a complementary approach to understanding health in the past. Finding ancient parasites has proved important for analyzing structures related to waste management, such as latrines, cesspits and sewer drains. The aim of this study was to analyze the sediment in four cesspits from the early modern period (16th-18th century CE) in the city of Granada, Spain. After rehydration, homogenization, and micro-sieving (RHM) with subsequent visualization under optical microscopy, roundworm (*Ascaris* sp.) and whipworm (*Trichuris* sp.) eggs were detected in all four cesspits, with liver fluke (*Fasciola* sp.) eggs also being found in one cesspit. These findings are consistent with written sources from this period, which describe waste management challenges as a cause of water contamination and reveal the possible utilization of human fecal material as fertilizer. The spread of parasites would have been favored by overcrowding in the city. This study offers the first analysis of cesspits from the early modern period in the Iberian Peninsula, and demonstrates that ineffective sanitation led to widespread infection of the population by intestinal worms.

1. Introduction

Paleoparasitology is defined as the study of parasites in paleontological and archaeological materials (Ferreira et al., 1979; Ferreira, 2014). Information on ancient parasites allows inferences to be drawn about the living conditions of past populations (Araújo et al., 2015), especially their health status, diet, hygiene, and organic waste management (Le Bailly et al., 2021; Mitchell, 2023). It can also improve our understanding of the evolution of host-parasite relationships (Hugot et al., 2022) and human-animal interactions (Ledger and Mitchell, 2022). Technological advances have enabled the detection of parasites in a wider range of materials (Camacho et al., 2020), including archaeological structures associated with waste management such as latrines, cesspits, and sewer drains (Love, 2007; Ledger et al., 2018; Chessa et al., 2020).

The most commonly found evidence for ancient parasites are the eggs of intestinal worms (helminths) (Araújo and Ferreira, 2000;

Bouchet et al., 2003). Their preservation in archaeological material is favored by the resistance of their shells to taphonomic processes (Wharton, 1980; Morrow et al., 2016), which are influenced by climate (Ramírez et al., 2022), environmental conditions (Camacho et al., 2016) and the composition of the soil (Rácz et al., 2015).

Ancient parasites have been described in a wide range of time periods (e.g. Le Bailly et al., 2014; Mitchell, 2015a, 2017a; Maicher et al., 2019). However, only limited research has been published on parasites in the early modern (16th-18th AD) and modern (18th-20th AD) periods in Europe (Anastasiou et al., 2012; Anastasiou, 2015; Graff et al., 2020; Gaeta and Fornaciari, 2022; Rabinow et al., 2023). This is especially true of the Iberian Peninsula, with only a few studies in Portugal (Sianto et al., 2017, 2018) and in Spain there is just a possible case of *Echinococcus granulosus* calcified cyst, *Trichinella spiralis* in the muscle of a mummified girl, and roundworm in the Canary Islands (Bellard and Cortés, 1991; Gijón-Botella et al., 2010; Monge-Calleja et al., 2017). Written sources from these periods also indicate the presence of

* Corresponding author.

E-mail address: ramonlopez131094@correo.ugr.es (R. López-Gijón).<https://doi.org/10.1016/j.jasrep.2023.104342>

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parasites (Carvalho, 2014; Mitchell, 2017b).

The main objective of this study was to determine the species of intestinal parasite that infected the population of southern Spain during the early modern period. We approached this through the analysis of four cesspits dating from 16th-18th centuries AD that were excavated at the archaeological site of Ventanilla Street in the city of Granada (Spain). The secondary objective was to compare our findings with documentary sources from the same period, including any descriptions of soil and water source contamination.

2. Material and methods

2.1. Archaeological site

The study material originated from an archaeological excavation in 2022 in Ventanilla Street in the city of Granada (Fig. 1). Modern, early modern, and medieval period layers could be clearly differentiated based upon the site stratigraphy (Suliman and Espinar-Moreno, 2022). The modern phase (19th and 20th centuries) had closed wastewater canals, remains of foundation walls, and bags of debris. The early modern phase (16th-18th centuries) shows remains of the walls of so-called “*corralas*” (corrals), buildings in which tenements on multiple floors surround a central courtyard, also known as “houses of neighbors” (Fig. 2). Four intact cesspits with different diameters and capacities were found within these walls (Fig. 3a and b). The medieval phase corresponds to the necropolis of Puerta Elvira (*maqbara* of Sahl Ben Malik), which was the main cemetery of the city from the 11th to 15th centuries and one of the most important in Al-Andalus (Charisi et al., 2016; López-López, 1998).

2.2. Historical context

The site under study was well documented in historical sources, being on the urban periphery of Granada during the 16th and 17th



Fig. 2. Example of a *corrala* (Corrala de Santiago) from the 17th century in Granada. Photo by University of Granada (Corrala de Santiago).

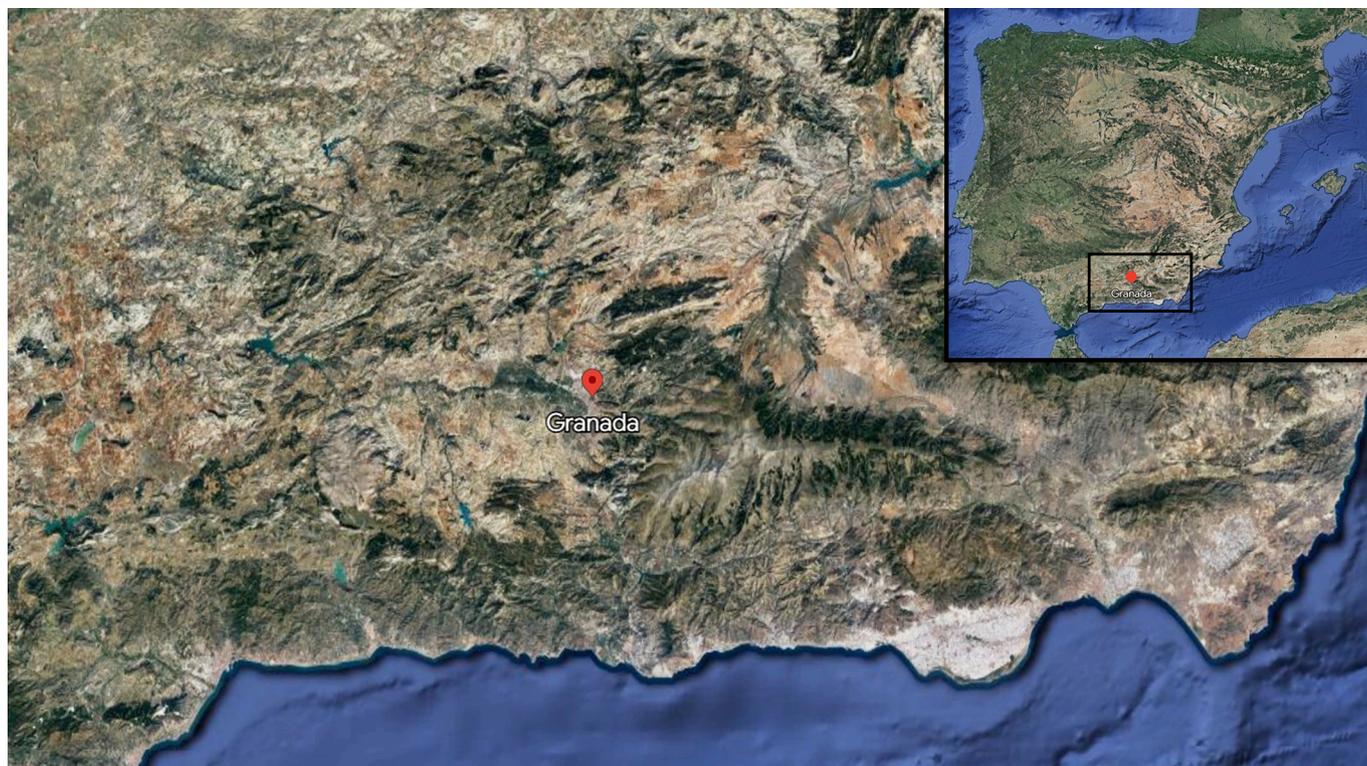


Fig. 1. Map showing the location of Ventanilla Street (Google Earth V 9.180.0.125 (April 16, 2023). Andalusian region, Granada, Spain. 37°10'55"N; 3°36'12"W, eye alt 247.03 km. Google 2023).

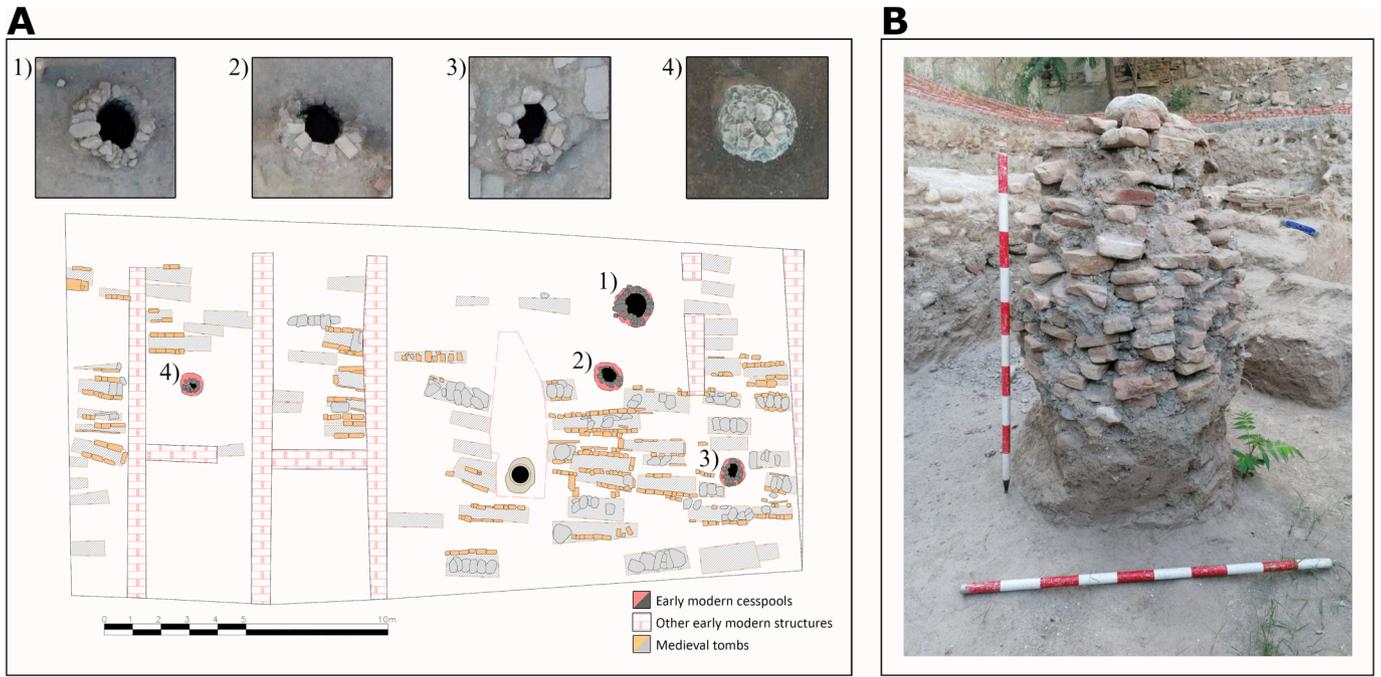


Fig. 3. A) ventanilla street in early modern and medieval phases. drawn by félix bizarro; b) intact cesspit found in ventanilla street. photo by ramón lópez-gijón.

centuries, representing a point of entry to the city from the surrounding countryside (Münzer, 1987). The name Ventanilla (“little window” in Spanish) appeared on maps since the 18th century and was related to the absence of windows with a view of neighboring San Jerónimo Monastery. This was ordered by Pedro de Baeza, the landowner in the 16th century. In the early 17th century the writer Henríquez de Jorquera

(1987) described the construction model of a new neighborhood, originally outside the city boundary, which included the San Juan de Dios Hospital and Fuente Nueva (New Spring), the main supply of water to the area. This neighborhood, divided between the parishes of San Ildefonso and Santos Justo y Pastor, was required as a result of population growth during the 17th and 18th centuries (Sánchez-Montes, 2000a).

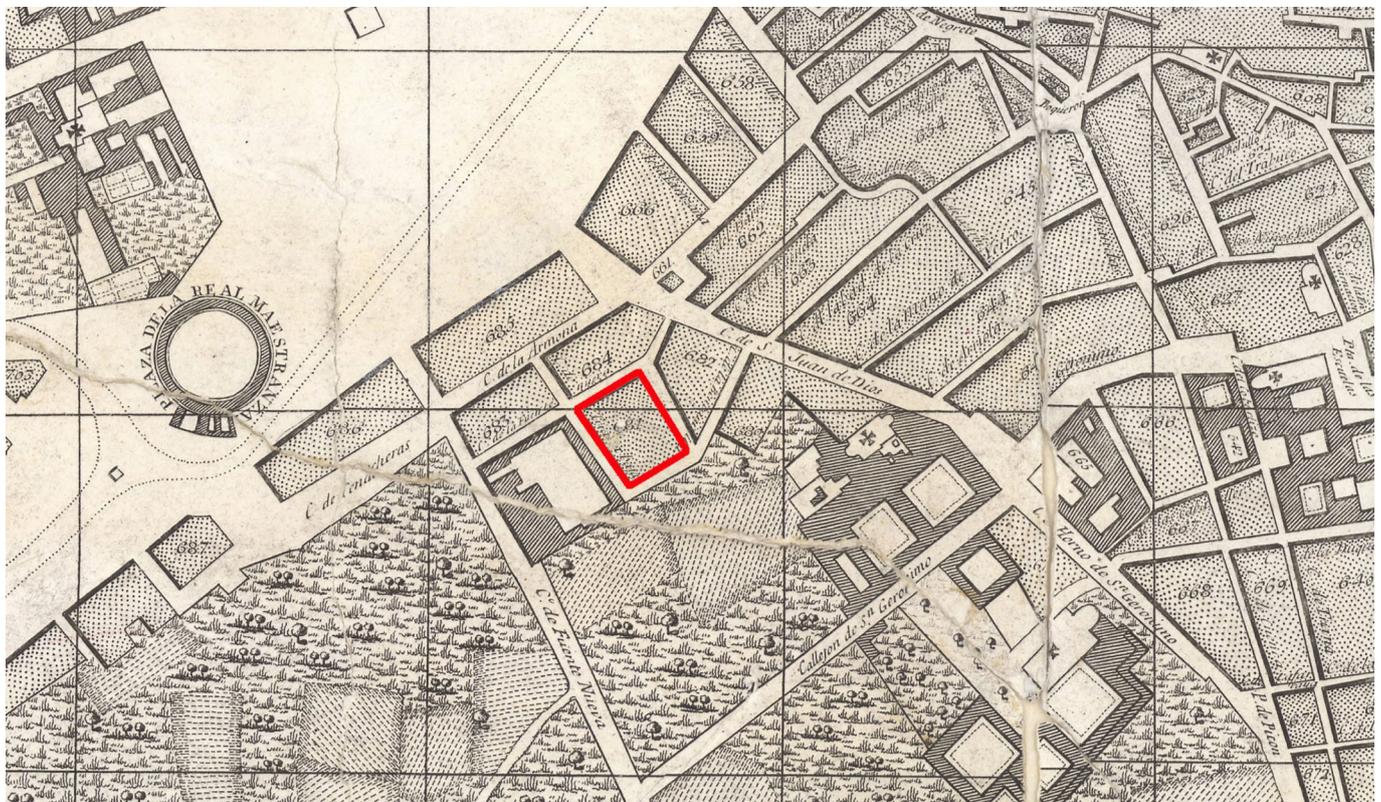


Fig. 4. Detail of Dalmau’s map printed 1796 showing the study site (Calatrava Escobar and Ruiz Morales, 2005). Edited by Pablo L. Fernández Romero and Salvatore Duras.

Unlike other urban spaces in the city where single family homes predominated, this new neighborhood was characterized by corrals in which multiple families lived. The aim was to make the metropolis more attractive to the expanding urban population (Sánchez-Montes, 2000b). It was located at one of the main entry points into the city for products from the surrounding fields, becoming known as the “barrio de la carretería” (neighborhood of carts). The topographic map by Francisco Dalmau in 1796 shows the three blocks of corrals (numbers 680 to 684), Fuentenueva Street, Ventanilla Street (Fig. 4), and cultivated land behind the church of San Juan de Dios.

The lifestyle of the population in this area was strongly influenced by the water from Fuentenueva spring. Its domestic role was evidenced by the discovery of wells in the corrals (which were fed by conduits from the Fuentenueva spring) and by uncovered channels (*acequias*) from other sources such as Alhacaba. In this way water for human consumption travelled through various conduits to the Fuentenueva basin, built in 1616 but long since lost. These also served as drinking troughs for animals, with consequent danger of human infection from the water. This contrasted with the belief by some people that the waters had medicinal properties (Henríquez de Jorquera, 1987).

2.3. Method

Paleoparasitological analysis was conducted in the four intact cesspits found at the archaeological site. Samples of their sediment were taken immediately upon opening the cesspits to avoid contamination of the material (Fugassa, 2014), with the excavator wearing dust-free nitrile gloves and surgical mask. Plastic spoons were used to gather the material, changing gloves and spoons after taking each sample to avoid cross-contamination.

The sampling methodology recommended by Le Bailly et al. (2021) was followed. Briefly, control samples (75–80 g each) were collected from outside each cesspit and from its upper “filling” layers. Samples were then gathered from each stratigraphic unit as defined by sediment color changes, dividing larger units (>25–30 cm) into artificial stratigraphic units (subunits), until the geological level was reached. The number of samples varied according to the capacity of each cesspit, gathering five samples each from cesspits 2 and 4, seven from cesspit 3 and eleven from cesspit 1. This gave a total of twenty-eight samples.

Each sample was labeled with the site name, site area, cesspit number, and stratigraphic unit and stored in a double plastic bag to avoid contamination. The material was transferred to the Laboratory of Physical and Forensic Anthropology of the University of Granada (Spain), where it was kept in darkness in controlled temperature and humidity to avoid degradation.

Laboratory analysis of the samples followed the Rehydration, Homogenization and Micro-sieving (RHM) protocol developed at the Université de Reims Champagne-Ardenne (France) (Bouchet et al., 2001) and perfected at the Université de Bourgogne Franche-Comté (France) (Dufour and Le Bailly, 2013). In brief, 5 g of each sample was rehydrated for seven days in aqueous solution containing 50 % 0.5 % trisodium phosphate (Na_3PO_4) and 50 % 5 % glycerol ($\text{C}_3\text{H}_8\text{O}_3$), with drops of 10 % formaldehyde (CH_2O) to prevent fungal growth. After rehydration and crushing in a porcelain mortar, the material was immersed in an ultrasound bath for 1 min and then passed through a tower of 315 μm , 160 μm , 50 μm , and 25 μm micro-sieves under a constant stream of water. Material trapped on the 50 μm and 25 μm micro-sieves were then collected. The contents were examined under light microscopy (Olympus CX43 at 100X, 400X, and 600X coupled to Olympus SC-50 camera), using Olympus CellSens software to process the images and analyzing 20 slides per sample ($n = 560$).

3. Results

The samples contained eggs of roundworm (*Ascaris* sp.), whipworm (*Trichuris* sp.) and liver fluke (*Fasciola* sp.), identified by their

morphology and egg dimensions (Table 1). *Ascaris* sp. was detected in all four cesspits between 1 and 8 eggs per sample, identified by their characteristic mamillated shell, oval shape, and dimensions (Fig. 5a). Samples also contained decorticated *Ascaris* sp. eggs (Fig. 5b), frequently found without their mamillated coat in paleoparasitological studies.

Trichuris sp. was also detected in all four cesspits at between 2 and 6 eggs per sample, identified by their bipolar protuberances (polar plugs), lemon shape, smooth surface, thick shell, and dimensions (Fig. 6).

Fasciola sp. was found in cesspit 1 (Fig. 7), identified by their flask shaped morphology with operculum at one end, dimensions, and characteristic thin shell.

4. Discussion

Ascaris sp. are found in humans (*A. lumbricoides*) and other mammals such as pigs (*A. suum*), while *Trichuris* sp. are observed in humans (*T. trichiura*), pigs (*T. suis*), and foxes (*T. vulpis*), with the possibility of human infection by the last two species (Roberts and Janovy, 2008; Betson et al., 2015). *Ascaris* sp. cannot be easily distinguished at a morphological (Leles et al., 2012) or genetic (Alves et al., 2016) level. In contrast, there are significant morphological differences between *T. vulpis* and *T. suis* or *T. trichiura*, but not between *T. trichiura* and *T. suis* (Betson et al., 2015). Due to this complexity, we will just refer to them here as roundworm and whipworm. While it is not possible to distinguish between the microscopic appearance of the highly similar eggs of different *Fasciola* sp. found across the world (Mas Coma et al., 2019), *F. hepatica* is by far the most commonly found *Fasciola* species in Europe.

The identification of parasite eggs in cesspits generally indicates the presence of parasite infection in the human population. However, it yields limited information on individual human infection because the original fecal material is mixed and not connected to biologically defined individuals. It is also possible that some taxa of parasites may be missing due to taphonomic action rather than the absence of parasitosis in humans (Ramírez et al., 2022). For instance, the preservation of parasite eggs is worse under arid rather than waterlogged conditions (Bouchet et al., 2003), and more parasite species have been reported in areas of the Iberian Peninsula with greater humidity (Maicher et al., 2017; López-Gijón et al., 2023) than in drier areas similar to the present site (Sianto et al., 2015; Knorr et al., 2019). The high fluctuations in temperature and precipitation typically experienced by the city of Granada may explain the small number of eggs retrieved (Rodrigo et al., 1999).

Samples from all cesspits analyzed contained the eggs of roundworm and whipworm. These geohelminths are commonly found in areas with poor sanitation and contaminated drinking water (Jourdan et al., 2018). Both parasites have a fecal-oral life cycle that is facilitated by ineffective hygienic-sanitary habits such as lack of hand washing (Fung and Cairncross, 2009), and the intake of water or food contaminated by human feces (Vaz Nery et al., 2019; Eslahi et al., 2022). The eggs of these geohelminths have especially resistant shells to protect them in the soil (Wharton, 1980), and they are produced in very high numbers. Thus, a roundworm female can lay up to 200,000 eggs/day and a whipworm female between 2,000 and 10,000 eggs/day (Roberts and Janovy, 2008). The tough egg shells and high worm fecundity explain why these are the two parasites most frequently detected in ancient European material (Anastasiou, 2015; Gaeta and Fornaciari, 2022).

The health consequences of these parasites depend on the number involved in the infection. Some infected individuals are asymptomatic, but others can experience diarrhoea, abdominal cramps, anaemia, malnutrition, stunted growth during childhood, reduced intelligence, and in severe roundworm infections intestinal obstruction may occur (Jourdan et al., 2018).

Liver fluke (*Fasciola* sp.) eggs were found in one of the cesspits. This zoonotic parasite infects the biliary tract of its host, usually herbivores such as ruminants. If herbivore liver is eaten the eggs will passed

Table 1
Details of the parasite eggs recovered from the four cesspits at Ventanilla Street.

Sample	<i>Ascaris</i> sp. Mean egg length in μm	<i>Ascaris</i> sp. Mean egg width in μm	Number of <i>Ascaris</i> sp. eggs	<i>Trichuris</i> sp. Mean egg length in μm	<i>Trichuris</i> sp. Mean egg width in μm	Number of <i>Trichuris</i> sp. eggs	<i>Fasciola</i> sp. Mean egg length in μm	<i>Fasciola</i> sp. Mean egg width in μm	Number of <i>Fasciola</i> sp. eggs
Cesspit 1C.S.	–	–	–	–	–	–	–	–	–
1 S.U. 1	–	–	–	–	–	–	–	–	–
1 S.U. 2	–	–	–	–	–	–	–	–	–
1 S.U. 3	–	–	–	–	–	–	–	–	–
1 S.U. 4	–	–	–	–	–	–	–	–	–
1 S.U. 5	–	–	–	–	–	–	–	–	–
1 S.U. 6	–	–	–	–	–	–	–	–	–
1 S.U. 7	62.25 (± 0.47)	44.39 (± 0.65)	3	–	–	–	–	–	–
1 S.U. 8	66.51 (± 2.27)	46.52 (± 1.38)	5	–	–	–	–	–	–
1 S.U. 8a	69.92 (± 1.16)	48.91 (± 1.12)	4	–	–	–	130.75 (± 7.54)	85.08 (± 4.14)	3
1 S.U. 8b	60.57 (± 1.34)	45.65 (± 1.31)	8	51.59 (± 0.84)	26.82 (± 0.74)	6	–	–	–
Cesspit 2C.S.	–	–	–	–	–	–	–	–	–
2 S.U. 1	–	–	–	–	–	–	–	–	–
2 S.U. 2	–	–	–	–	–	–	–	–	–
2 S.U. 3	61.81	44.38	1	51.43 (± 0.43)	25.53 (± 0.46)	4	–	–	–
2 S.U. 4	59.09 (± 2.24)	46.94 (± 1.51)	2	52.42 (± 1.76)	26.54 (± 0.15)	3	–	–	–
Cesspit 3C.S.	–	–	–	–	–	–	–	–	–
3 S.U. 1	–	–	–	–	–	–	–	–	–
3 S.U. 2	67.48 (± 2.04)	47.35 (± 2.37)	6	50.68 (± 0.2)	25.27 (± 1.42)	3	–	–	–
3 S.U. 3	62.62 (± 0.74)	46.67 (± 1.22)	8	49.62 (± 1.44)	25.08 (± 0.49)	4	–	–	–
3 S.U. 4	61.90 (± 1.88)	44.28 (± 1.79)	4	50.62 (± 0.51)	25.08 (± 0.58)	2	–	–	–
3 S.U. 5	63.84 (± 1.44)	48.49 (± 1.17)	4	51.86 (± 0.75)	25.66 (± 0.61)	3	–	–	–
3 S.U. 5a	66.21 (± 1.70)	49.07 (± 3.08)	3	50.87 (± 1.58)	24.72 (± 0.47)	3	–	–	–
Cesspit 4C.S.	–	–	–	–	–	–	–	–	–
4 S.U. 1	–	–	–	–	–	–	–	–	–
4 S.U. 2	–	–	–	–	–	–	–	–	–
4 S.U. 3	61.74 (± 0.97)	43.82 (± 1.21)	3	52.13 (± 1.55)	27.33 (± 0.64)	4	–	–	–
4 S.U. 4	62.28 (± 1.32)	44.63 (± 1.49)	4	49.90 (± 0.72)	25.32 (± 0.55)	5	–	–	–

harmlessly though the intestines to end up in the human feces, but not indicate human infection. However, humans can be genuinely infected by consuming vegetables or water contaminated by *Fasciola* metacercariae (Cwiklinski et al., 2016; Mas-Coma et al., 2019). This is in contrast to the mode of transmission of the geohelminths roundworm and whipworm. Mild infections can be asymptomatic, while more severe infections can cause fever, nausea, abdominal pain, hepatomegaly, weight loss, and anemia (Lalor et al., 2021). Its presence in the environment would indicate a potential risk of human infection by this parasite (Wang and Mitchell, 2022).

The only one of these parasites that appears in contemporary Spanish texts is roundworm, which was mentioned by Miguel de Agustín in the 17th century and by Lorenzo Hervás y Panduro in the 18th century (Cordero del Campillo, 1980).

There is limited evidence of parasites in cesspits, latrines, or sewer drains from the early modern period in Europe (Anastasiou, 2015), but a few studies have been conducted in the UK (Anastasiou et al., 2012; Ryan et al., 2022), Belgium (Fernandes et al., 2005; Rocha et al., 2006; Graff et al., 2020; Rabinow et al., 2023), the Czech Republic (Bartošová et al., 2011), and France (Bouchet et al., 1998).

The prevalence of parasite eggs in the environment can be markedly increased by the ineffective management of human waste, especially by

its utilization to fertilize crops. Paleoparasitology studies have evidenced the persistence of this practice throughout history, (Mitchell, 2015b; Dufour et al., 2016; Knorr et al., 2019; Langgut, 2022). The proximity of the cesspits in Ventanilla Street to surrounding fields (often fertilized with fecal waste), the passage through the area of agricultural produce and cattle, and deficient waste management would all have increased the risk of parasitosis in the population. These factors, alongside the use of Fuentenueva waters by both humans and animals, would have favored the outbreak of epidemics by intestinal microorganisms. Indeed, Granada suffered from various mortality crises during the 17th century, such as the bubonic plague epidemic in 1679 (Jiménez-Brobeil and Al Oumaoui, 2002), and the second most severe crisis being an epidemic propagated by fecal-oral transmission in 1648. The parish mortality records from some parts of the city suggest that the epidemic resulted from contamination of certain watercourses (Jiménez-Brobeil et al., 2020). The Granada Council attempted to address this problem in the 17th century with the promulgation of Water Ordinances (*Ordenanzas de las Aguas*) requiring water conduits, wastewater canals, and the river to be cleaned in March and September every year. It was also forbidden to throw human waste or dead dogs, cats, or chickens into the street (which carried a fine of 3000 maravedies), or to wash clothes or fish there (Granada Council, 1670). The need for these

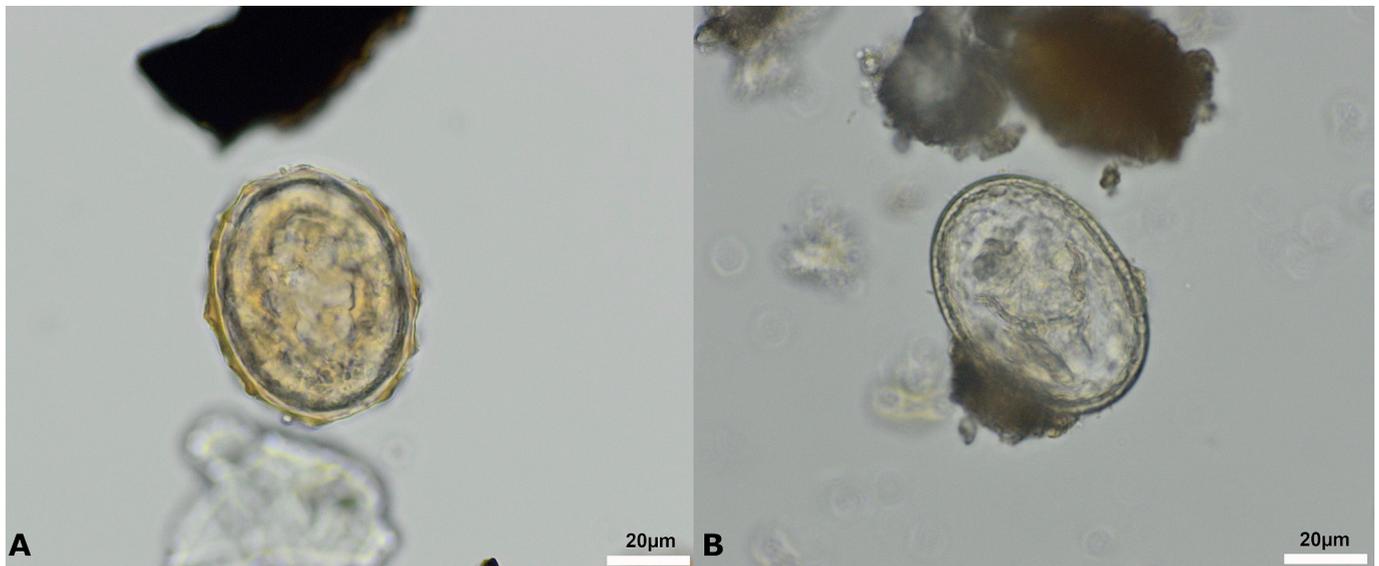


Fig. 5. A) *Ascaris* sp. with mamillated coat intact (61x 49 µm); b) Decorticated *Ascaris* sp. (63 x 45 µm). Photo by Ramón López-Gijón.



Fig. 6. *Trichuris* sp. (49 x 26 µm). Photo by Ramón López-Gijón.

regulations indicates the scale of the sanitary problems faced by the city, which would have been exacerbated by the utilization of dirty water from irrigation canals in houses and vegetable gardens. Water contamination is known to be a risk factor for parasitosis due to geohelminths (Bowman, 2021; WHO, 2023) and liver flukes (Sabourin et al., 2018; Mas-Coma et al., 2018), as observed in The Netherlands, Denmark, and Lithuania (Søe et al., 2018).

Overcrowding plays a major role in the spread of parasites such as roundworm and whipworm (Scott, 2008; Parija et al., 2017). The cesspits in this study were in an area of high population density, where groups of neighbors lived together in *corralas* (Sampelayo, 1980). This overcrowding represents a further risk factor for parasitosis in this

population (Roche et al., 2021).

5. Conclusion

This study contributes the first evidence from the Iberian Peninsula for the presence of parasites in cesspits in use during the early modern period. We found the eggs of roundworm, whipworm, and liver fluke in the city of Granada during the 16th, 17th, and 18th centuries. Their presence is attributable to environmental and food contamination, poor-quality drinking water, and the utilization of human fecal material as fertilizer. Contemporaneous documentary sources describe infections caused by fecal-oral transmission in epidemics that were sometimes

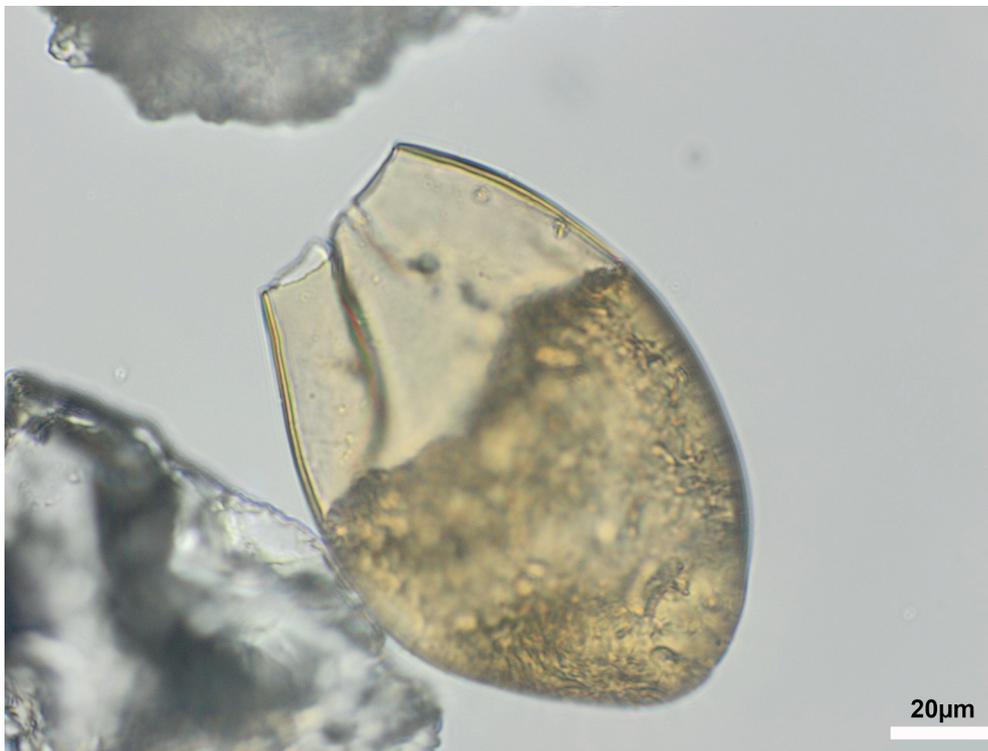


Fig. 7. *Fasciola* sp. egg with operculum lost (123 x 79 μ m). Photo by Ramón López-Gijón.

responsible for mortality crises. The limited number of parasite eggs may be attributable to taphonomic action, including the negative effect of the local climate upon egg preservation. This study lays the foundations for future parasite research in the region of Granada and contributes to our knowledge of parasites in Europe during the early modern period, which has received limited attention in the past.

CRediT authorship contribution statement

Ramón López-Gijón: . Sylvia Jiménez-Brobeil: Funding acquisition, Project administration, Resources, Writing – review & editing. **Rosa Maroto-Benavides:** Funding acquisition, Project administration, Resources. **Salvatore Duras:** . **Amjad Suliman:** . **Pablo L. Fernández Romero:** Investigation. **Miguel C. Botella-López:** Writing – review & editing. **Francisco Sánchez-Montes:** Investigation. **Piers D. Mitchell:** .

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

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