International Journal of Paleopathology 41 (2023) 55–58

Contents lists available at ScienceDirect

International Journal of Paleopathology

journal homepage: www.elsevier.com/locate/ijpp

Brief communication

Zoonotic parasite infection from a funerary context: A Late Antique child case from Cantabrian Spain

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ARTICLE INFO

Keywords:
Paleoparasitology
Pseudoparasitology
Human-animal interaction
Dicrocoelium

ABSTRACT

Objective: To evaluate the presence of *Dicrocoelium* sp. in a child from a Late Antique funerary context from Cantabrian Spain and discuss whether the infection is true infection or pseudoparasitosis.
Materials: Four skeletons, including one from a 5–7 year old child, have been analysed from the archaeological site of El Conventón, dated between the sixth and seventh centuries AD.
Methods: The paleoparasitological study was conducted through the analysis of soil samples from different parts of the skeleton and funerary context using the rehydration, homogenization, and micro-sieving method, and visualized through brightfield microscopy.
Results: A soil sample from the pelvic region tested positive for *Dicrocoelium* sp. (possibly *D. dendriticum*).
Conclusions: The child was infected with *Dicrocoelium dendriticum,* which based on archaeological and historical contexts may be related to hygiene or dietary behaviour.
Significance: We present one of the few cases of the identification of a Dicrocoelidae parasite directly associated with a human skeleton that provides historical knowledge of a zoonotic disease.
Limitations: The diagnosis of a zoonosis through the identification of ancient parasites is complex. In addition, *Dicrocoelium* sp. in association with skeletal human remains is rare due to the potential low prevalence of this parasite.
Suggestions for Further Research: Highlight the importance of paleoparasitological analysis to link parasitic infection diseases with socioeconomic issues by using funerary contexts with skeletal remains.

1. Introduction

Specific parasites, linked to zoonotic infections, are key pathogens used to reconstruct human-animal interactions in the present and the past. Their presence is closely linked to evolutionary, cultural and ecological factors (Ledger and Mitchell, 2019). Among these parasites is a trematode in the genus *Dicrocoelium*, which can zoonotically infect humans, but mainly infects bovids in the Holarctic region (i.e., *D. dendriticum*) (Mowlavi et al., 2015). Although infection by *D. dendriticum* (aka lancet liver fluke) is evidenced in the past, especially during the Roman and Mediaeval periods (Bouchet et al., 2003; Le Bailly and Bouchet, 2010), this zoonosis is not prevalent, as humans rarely enter the biological cycle of this parasite (Roche et al., 2021). This is also the situation for modern clinical cases (Magi et al., 2009). Furthermore, this zoonotic disease may be acquired as a true infection (i.e., direct consumption of ants, leading to harboring the parasite in the human liver (Searcey et al., 2013), or as a pseudo-infection through consumption of infected animal liver (Mowlavi et al., 2015; Roche et al., 2021). Distinguishing between these two modes of infection is difficult when the archaeological evidence is derived from faeces (Bouchet et al., 2003) or sediment samples. However, the presence of these parasites is key to understanding human-animal interactions such as animal management and dietary habits in the past (Le Bailly and Bouchet, 2010).

Here, we present the paleoparasitological analysis of four tombs from the Visigothic cemetery of El Conventón (Cantabrian Spain) dated between the sixth and seventh centuries AD. One of the individuals, a child aged 5–7 years old, showed a positive determination for the parasite *Dicrocoelium* sp. (possibly *D. dendriticum*), providing the opportunity to evaluate health issues related to dietary behaviour and hygienic conditions during the Late Antiquity-Early Mediaeval period in

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https://doi.org/10.1016/j.ijpp.2023.03.003
Received 9 December 2022; Received in revised form 6 March 2023; Accepted 7 March 2023
Available online 3 April 2023

Fig. 1. a) Location of the site in Cantabrian Spain; b) El Conventón site with the Roman, Late Antique Visigothic and Mediaeval phases; c) Funerary context of the four individuals analysed; and d) Sampling for parasitological analysis. Planimetry (b) and photo (c) are modified and used with permission and courtesy of L. Mantecón and P. Fernández-Vega.

Northern Iberian Peninsula. Furthermore, the diagnosis of this zoonotic disease allows us to reconstruct specific human-animal interactions. This case represents the first evidence of the identification of this parasite associated with human skeletal remains in the Iberian Peninsula, although the presence of *Dicrocoelium* has been previously identified archaeologically since the Neolithic (Maicher et al., 2017).

2. Materials and method

2.1. The archaeological site and funerary context

The cemetery of El Conventón is located in the archaeological site of Camesa-Rebolledo (Cantabria, Spain) (Fig. 1a). The Late Antique inhumations, dating from the sixth to the seventh centuries AD, are mostly simple graves dug through the remains of a previous Roman house (Fig. 1b) (Gutiérrez-Cuenca, 2015; Carnicero, 2015). This interval during Late Antiquity is known as the Visigothic period, when the re-utilisation of Roman structures as burial spaces (necropolis) is common in northern Iberian rural areas after the collapse of the Roman authority. Later mediaeval churches were built next to nearby villages in a well-documented settlement pattern (Fernández, 2013), as in the case of El Conventón site.

Four Late Antique graves were studied. They are located in Square GB101 from Level 2 and belong to four individuals: Individual 1, an adult male; Individual 2, an adult female; Individual 4, an adult of indeterminate sex; and Individual 3, a child (see Fig. 1c and Supplemental Material Fig. S1) (Carnicero, 2015). The child was aged 6–7 years old based on to the tooth development and eruption and 5–7 years old based on the length of the clavicle (see White and Folkens, 2005). The sex of the child was not determined and no pathological lesions were observed. The funerary conditions include burial in an extended supine position with decomposition in a void space (Knüsel and Schotsmans, 2022).

2.2. Paleoparasitological analysis

The paleoparasitological study was conducted using five grams of soil samples taken in situ from different areas of the tombs and skeletons (see Fig. 1d and Supplemental Material Fig. S1). Avoiding contamination, samples were taken from the pelvic and thoracic areas, in addition to cranial and feet zones as control samples (total n = 16 samples) (e.g., Fig. 2a and Supplemental Material Fig. S1). Analysis followed the RHM protocol (rehydration, homogenization, and micro-sieving) after Dufour and Le Bailly (2013). This protocol is an extraction method based on the micrometric separation of elements using a four mesh column (315, 160, 50, 25 μm). The filtered content was analysed using light microscopy (Olympus CX43RF at 100, 400 and 600 magnifications and camera Olympus SC-50. The software Olympus CellSens was used to process the images). For each sample, 20 slides were analysed (n = 320).

3. Results

A total of 320 slides were analysed from 16 soil samples. No eggs were observed in all control samples, and thoracic and pelvic samples for individuals 1, 2 and 4. Individual 3 (sample 3.3 in Fig. 2a and Supplemental Material Fig. S1) demonstrated the presence of one structure consistent morphologically and in size (34.89 μm in length and 24.70 μm in breath) with a *Dicrocoelium* sp. egg (Fig. 2b and c). The egg can be characterised as dark brown in colour, thick-shelled, and operculated at one pole. This is compatible with previous *Dicrocelium* sp. identifications (Mas-Coma and Bargues, 1997; Roberts and Janovy, 2008). The rarity of the remains (0–1 egg) can be explained by the source of the samples (i.e., from skeletal remains).

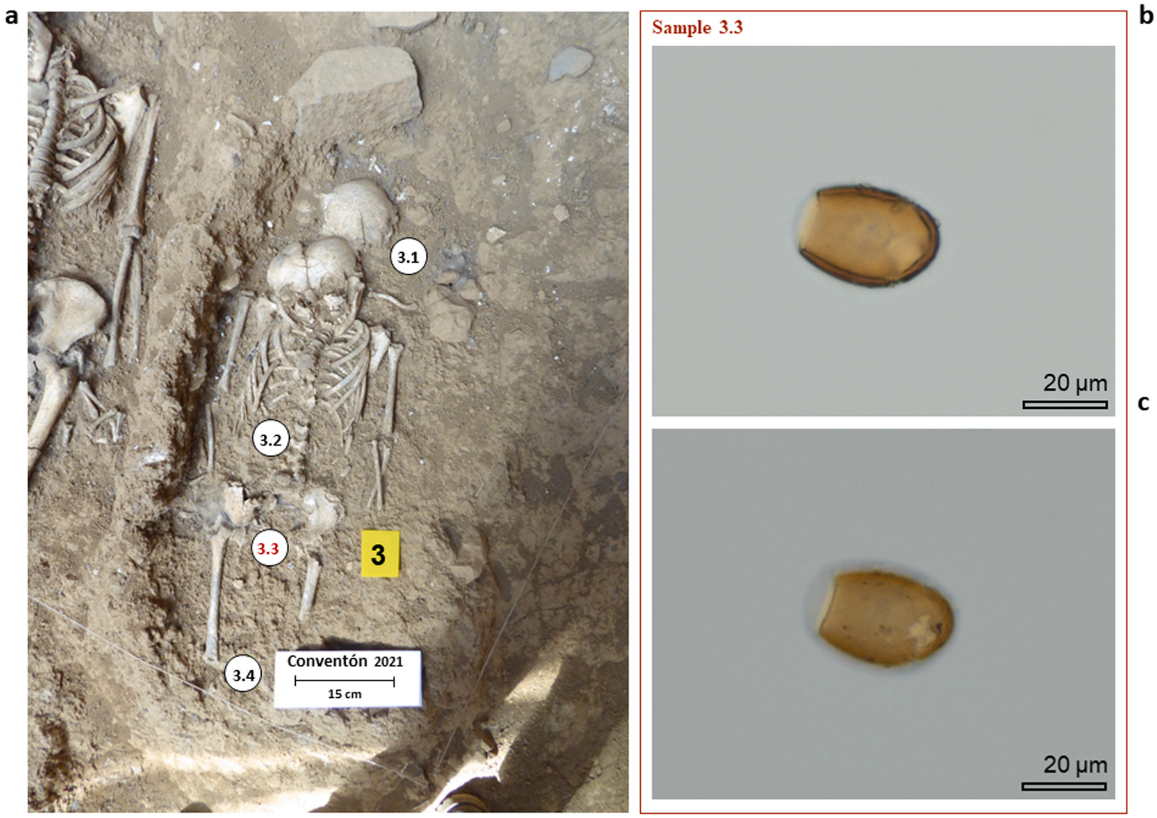

Fig. 2. a) Location where soil samples were taken from Individual 3. Of the four areas, labeled 3.1, 3.2. 3.3, and 3.4, only in area 3.3 was the *Dicrocelium* sp. parasite egg identified; b) section of the *Dicrocelium* sp. parasite egg (possibly *D. dendriticum*); c) surface view of the recovered parasite egg.

4. Conclusion and future perspectives

This case study presents a possible zoonotic infection of a 5–7 year old child with dicrocoeliasis, during Late Antiquity (6th-7th century AD), representing the first case of *Dicrocoelium* sp. parasite identification associated with human skeletal remains in the Iberian Peninsula. Given the European geographical location and morphology of the identified *Dicrocoelium* sp., it is suggested that this finding is *D. dendriticum,* which is in accord with the findings of Le Bailly and Bouchet (2010). Furthermore, the spatial association between the identified pathogen and the skeleton provides an anatomical location that allows us to suggest a direct relationship between host and parasite.

However, the source of the zoonotic infection is difficult to determine. The infection might be a true human infection (i.e., ingestion of infected ants directly or indirectly from geophagy or foodborne), or acquired as a pseudoparasitic infection (i.e., the consumption of infected liver from other animals, especially bovids, or from goat, sheep and deer based on Roberts and Janovy, 2008). On the one hand, true infection is generally rare in both the past and the present (Magi et al., 2009), although it was identified in a Roman bog human mummy, due to the preservation of the liver (Searcey et al., 2013). On the other hand, pseudoparasitism is a much more common scenario (Mowlavi et al., 2015) mainly linked to the consumption of raw or undercooked animal liver (Reinhard et al., 2013; Araújo et al., 2015). Although our case may reflect a true infection related to direct or indirect ingestion of ants, we assume that pseudoparasitism is a more reliable possibility. Hence, a plausible explanation for the infection could be linked to culinary habits that included the intake of raw or undercooked bovid liver. Perhaps, animal liver was one of the few sources of iron for children, as it has been for women in the past (Bullough and Campbell, 1980). Since there is no evidence to relate our case to iron deficiency, consuming liver could have been part of the diet in Late Antique rural Cantabria, which was mainly dedicated to agricultural activities and husbandry of a variety of domestic animals (Gutiérrez-Cuenca, 2015.

The identification of the parasite *D. dendriticum* associated with human skeletal remains is rare in the archaeological record. However, given the importance of this pathogen for understanding human-animal interactions in the past, paleopathology ought to direct efforts towards comparative paleoparasitological analyses. The aim of the present case is to provide comparative data, in addition to highlighting the importance of the study of ancient parasites within human osteoarchaeological contexts in order to understand health in the past.

Funding

This research received funding from the European Union's Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement TRAUMOBITA No 895712.

Declaration of Competing Interest

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

Acknowledgements

We express our gratitude to the staff working at the Camesa-Rebolledo Site Museum (Centros Culturales de Cantabria - Gobierno de Cantabria) for their support during our fieldwork sampling. This research is part of RLG's PhD dissertation, and we acknowledge the use of King's College Cambridge facilities to write this paper during a research stay by RLG. We appreciate the useful comments by the IJP Editors and the anonymous reviewers.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.ijpp.2023.03.003.

References

Araújo, A., Reinhard, K., Ferreira, L.F., 2015. Palaeoparasitology - human parasites in ancient material. Adv. Parasitol. 90, 349–387. https://doi.org/10.1016/bs.apar.2015.03.003.

Bouchet, F., Guidon, N., Dittmar, K., Harter, S., Ferreira, L.F., Chaves, S.M., Reinhard, K., Araújo, A., 2003. Parasite remains in archaeological sites. Mem. Inst. Oswaldo Cruz 98, 47–52. https://doi.org/10.1590/S0074-02762003000900009.

Bullough, V., Campbell, C., 1980. Female longevity and diet in the Middle Ages. Speculum 55, 317–325.

Carnicero, S., 2015. Antropología física de las poblaciones antiguas en Cantabria. Sautuola: Rev. Del. Inst. De. Prehist. Y. Arqueol. Sautuola 20, 387–400.

Dufour, B., Le Bailly, M., 2013. Testing new parasite egg extraction methods in paleoparasitology and an attempt at quantification. Int. J. Paleopathol. 3, 199–203. https://doi.org/10.1016/j.ijpp.2013.03.008.

Fernández, D., 2013. City and Countryside in Late Antique Iberia. Antiq. Tardive 21, 233–241. https://doi.org/10.1484/J.AT.5.101414.

Gutiérrez-Cuenca, E., 2015. Génesis y evolución del cementerio medieval en Cantabria. (Doctoral dissertation). University of Cantabria, Santander. hdl.handle.net/10902/7410.

Knüsel, C.J., Schotsmans, E.M. (Eds.). 2022. *The Routledge Handbook of Archaeothanatology: Bioarchaeology of Mortuary Behaviour*. Routledge.

Le Bailly, M., Bouchet, F., 2010. Ancient dicrocoeliosis: occurrence, distribution and migration. Acta Trop. 115, 175–180. https://doi.org/10.1016/j.actatropica.2010.03.004.

Ledger, M.L., Mitchell, P.D., 2019. Tracing zoonotic parasite infections throughout human evolution. Int. J. Osteoarchaeol 32, 553–564. https://doi.org/10.1002/oa.2786.

Magi, B., Frati, E., Bernini, L., Sansoni, A., Zanelli, G., 2009. *Dicrocoelium dendriticum*: a true infection. Infez. Med. 17, 115–116.

Maicher, C., Hoffmann, A., Côté, N.M., Palomo Pérez, A., Saña Segui, M., Le Bailly, M., 2017. Paleoparasitological investigations on the Neolithic lakeside settlement of La Draga (Lake Banyoles, Spain). Holocene 27, 1659–1668. https://doi.org/10.1177/0959683617702236.

Mas-Coma, S., Bargues, M., 1997. Human liver flukes: a review. Res. Rev. Parasitol. 57, 145–218.

Mowlavi, G., Mokhtarian, K., Makki, M.S., Mobedi, I., Masoumian, M., Naseri, R., Hoseini, G., Nekouei, P., Mas-Coma, S., 2015. Dicrocoelium dendriticum found in a Bronze Age cemetery in western Iran in the pre-Persepolis period: the oldest Asian palaeofinding in the present human infection hottest spot region. Parasitol. Int. 64, 251–255. https://doi.org/10.1016/j.parint.2015.02.007.

Reinhard, K.J., Ferreira, L.F., Bouchet, F., Sianto, L., Dutra, J.M., Iniguez, A., Leles, D., Le Bailly, M., Fugassa, M., Pucu, E., 2013. Food, parasites, and epidemiological transitions: a broad perspective. Int. J. Paleopathol. 3, 150–157. https://doi.org/10.1016/j.ijpp.2013.05.003.

Roberts, L.S., Janovy Jr, J., 2008. Foundations of Parasitology, eighth ed. McGraw Hill.

Roche, K., Capelli, N., Pacciani, E., Lelli, P., Pallecchi, P., Bianucci, R., Le Bailly, M., 2021. Gastrointestinal parasite burden in 4th-5th c. CE Florence highlighted by microscopy and paleogenetics. Infect. Genet. Evol. 90, 104713 https://doi.org/10.1016/j.meegid.2021.104713.

Searcey, N., Reinhard, K.J., Egarter-Vigl, E., Maixner, F., Piombino-Mascali, D., Zink, A. R., van Der Sanden, W., Gardner, S.L., Bianucci, R., 2013. Parasitism of the zweeloo woman: dicrocoeliasis evidenced in a roman period bog mummy. Int. J. Paleopathol. 3, 224–228. https://doi.org/10.1016/j.ijpp.2013.05.006.

White, T.D., Folkens, P.A., 2005. The Human Bone Manual. Elsevier.