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Research article

A possible *Echinococcus granulosus* calcified cyst found in a medieval adult female from the churchyard of Santo Domingo de Silos (Prádena del Rincón, Madrid, Spain)

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ABSTRACT

Calcification, or mineralisation, can occur as part of a natural process, or by pathological processes. The purpose of this work is to examine an unidentified semi-spherical and perforate hollow mass, found near the pelvis of an adult female, dated 12th–13th century AD, exhumed of the Church of Santo Domingo de Silos (Prádena del Ricón, Madrid, Spain).

The mass was examined by SEM and Energy Dispersive X-Ray Spectroscopy. These procedures revealed a heterogeneous inner surface with both smooth and irregular areas. A larger spherical and several smaller crescent-shaped perforations were noticed. X-ray microanalysis revealed the presence of the elements C, K, P, Ca, Al, Si, Fe, and Mg. The co-localisation of Ca and P suggests that they may be combined in a mineral matrix, likely formed *in vivo*. Other minerals probably came from the soil, although Fe could be related to the presence of blood. The macroscopic and microscopic appearances, chemical composition, and location of the calcified mass point to a possible hydatid cyst from *Echinococcus granulosus*, common in agricultural populations. This study used a suite of analytical techniques that are useful in the diagnosis of unknown calcified masses and can, therefore, be recommended for use in future analytical work.

1. Introduction

Calcification is a process shared by organisms such as plants, invertebrates, and vertebrates (Pautard, 1965). In the latter, this event can be part of a natural process – bone formation, better designated as ossification – or be triggered by pathological processes with soft tissue involvement (Waters-Rist et al., 2014). Both natural and pathological mineralisation mostly depend on calcium phosphate salt precipitation, with a small amount of inorganic ions (Pautard, 1965; Ramírez et al., 2004), having in common the presence of matrix vesicles as a nucleation core (Boskey, 1981).

The term "calcification" is a generic designation for all types of growth that occur outside bones and contain calcium (Bazin et al., 2014). When these deposits are arranged in areas that are external to bones, they are considered to be pathologic (Schoen, 1987). Although the majority of calcifications are asymptomatic, if they expand they can hamper the correct function of an organ (Lamb et al., 1991). Cysts are sac-like structures filled with liquid, soft, or (rarely) solid substances (Manuila et al., 2004) that usually affect soft tissues (Ramírez et al., 2004) and can be a secondary effect of implants (Schoen, 1987) or parasitic and/or bacterial infections (Carson, 1998; Waters-Rist et al., 2014), can be provoked by tumours (Theuwissen et al., 2012), or result from aging with ligament calcifications (Ramírez et al., 2004).

The literature currently highlights three types of calcifications: concretions or formations found in the lumen of an organ (Baker, 2015), and dystrophic and metastatic calcifications (Bazin et al., 2012), which are ectopic growths (Cotran et al., 1999). Dystrophic calcifications result more frequently from an injury or tissue necrosis, with normal concentrations of calcium (Baker, 2015), while metastatic calcifications provoke mineral disturbance (Meyers, 1995). In some tumours, the mineralisation process is triggered

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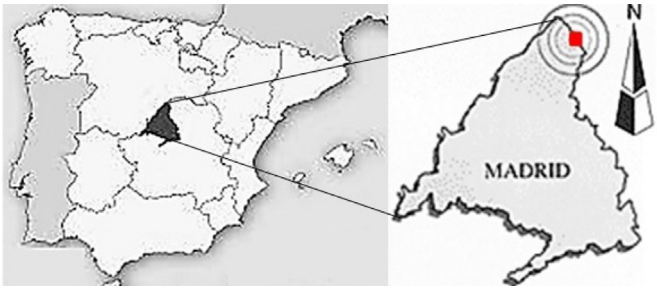

Fig. 1. Map showing the location of Prádena del Rincón in the province of Madrid, Spain.

by an increase of internal cellular pH associated with an impaired metabolism (Kunieda et al., 1990; Theuwissen et al., 2012), as well as by the segregation of macromolecules with a high affinity for calcium (Cotran et al., 1999). When a calcified mass results from a parasite, there are more questions about cyst calcification than answers. Their evaluation from an anthropological perspective may allow for a good reconstruction of both the distribution pattern of an infectious disease and the interaction established between the parasite and the host (Mitchell, 2013). Endoparasitic remains may be resistant to host reactions (Ortner, 2011) and their outermost layers can survive in the burial environment. Therefore, in an excavation one must be extremely careful (Mitchell, 2013; Sianto and Santos, 2014) while identifying this fragile mass.

In clinics, most information about parasitic calcified masses is obtained by radiological examinations in which variables such as appearance, number, pattern, location, size, shape, distribution, and density (Ramírez et al., 2004) can be used for diagnosis. However, this technique is not very suitable for establishing the nosology of abnormal growths (Baker, 2015) and has been gradually replaced by computerised tomography (Saba et al., 2009). In an archaeological context, diagnosis is even more difficult (Komar and Buikstra, 2003; Armentano et al., 2012).

In paleopathological studies, evidence of calcifications is scarce and, in most cases, the range of possible diagnoses is extremely wide (see Komar and Buikstra, 2003; Perry et al., 2008). The location of the mass is important for the diagnosis; however, body decomposition may induce displacement of the abnormal mass. At the same time, the uncertainty surrounding the mechanism by which inorganic salts precipitate, as part of the process of mineralisation, greatly affects the differential diagnosis. During macroscopic observation, three basic variables are commonly reported: morphology, robustness, and the absence or presence of vascularisation (Komar and Buikstra, 2003). The existence of blood vessels is crucial to narrowing the list of ectopic growths: tumour growths are more vascularised, while parasitic cysts are less vascularised (Komar and Buikstra, 2003). Microtomography is a critical diagnostic tool, allowing the observation of inner layers without damaging the sample.

This study aims to describe the macroscopic and microscopic properties of a calcified formation found near a female skeleton, and to relate this finding to the historical and social context of the medieval settlement of Prádena del Rincón.

2. Material and methods

2.1. Historical background

Prádena del Rincón, located in the surroundings of Madrid (Fig. 1), was one of the multiple livestock centres located near around worship areas (Cano Martín, 2010) and burial grounds that were built as part of the repopulation process associated with the Spanish *Reconquer* (Álvarez Palenzuela, 2002). The church of Santo Domingo de Silos was built over a pre-existing graveyard, in order to sanctify the space, leaving the graves outside the building in accordance with the Catholic prohibition of burial inside a consecrated construction (Fernández Grueso, 2007). This rule ended at the end of the 13th century, coinciding with the annexation of the northern atrium and its use as a burial place until the 15th century (Cano Martín, 2010). According to the church's obituary record book, since the 15th century, the atrium cemetery has been used exclusively for burial of children and paupers (Cano Martín, 2010).

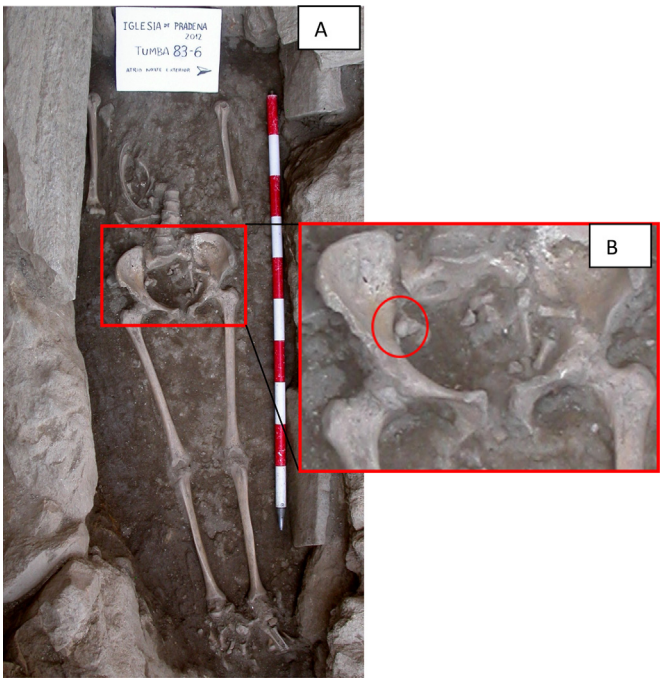

Fig. 2. Anatomical disposition of individual sk. 83-6. A. The skull and bones of the superior part of the skeleton are missing. B. Close up of the pelvic area, showing a strange mass on the right side (circle) and the hand bones.

During the restoration work of the church of Santo Domingo de Silos, performed between 2010 and 2012, a total of 90 medieval graves were discovered, dating between the 12th and 15th centuries AD (Cano Martín, 2010). According to this author, 64 graves were located in the external access of the northern atrium, carved into the rock and closed by slabs. The supine position and east-west orientation of the bodies, along with the crossing of the arms over the chest or abdomen, and the outstretched lower limbs, suggest a Christian inhumation (Lane, 2001). The pressure on existing burial spaces resulted in the overlap and reuse of graves, as well as the relocation of the skeletons into ossuaries (Cano Martín, 2010). The anthropological examination of bone remains revealed a Minimum Number of Individuals of 245, of which 125 (51%) corresponded to non-adults (Herrerín et al., 2016). Due to the fragmentary nature of the remains, sex could only be diagnosed for 43 adults—23 males and 20 females (Herrerín et al., 2016).

2.2. The sample

The individual sk. 83-6 object of this study (Fig. 2) was inhumed in the area of the atrium that, according to historical and archaeological evidence, is dated between 12th and 13th centuries AD. This skeleton was placed at the bottom of grave 83, beneath five other individuals with more recent chronologies, each of them separated by a layer of soil.

During the laboratory observation, sk. 83-6, the individual was identified as a female, based on the morphological characteristics of the os coxae described by Bruzek (2002). The pubic symphysis