

Pre-Columbian Tuberculosis in Northwest Argentina: Skeletal Evidence from Rincón Chico 21 Cemetery

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ABSTRACT Systematic excavation of collective burial sites makes possible the recovery of skeletal series which may show bony evidence of infectious pathological conditions. This paper presents the first evidence of the existence of tuberculosis in prehistoric populations of NW Argentina with a subsistence economy based on agriculture and pastoralism. The study was carried out on individuals from Rincón Chico 21 cemetery, a burial site located in the Santa María Valley, Catamarca, used between the Late Ceramic Period and the onset of the Inca empire expansion (AD 1000–1400). Six individuals out of the 70 so far excavated showed destructive lesions in the vertebral bodies and periosteal reactions in other bones. The morphology and distribution of bone lesions led us to rule out several diseases from a broad spectrum of possible diseases that could have affected the skeletal system. Thus, the lesions were interpreted as caused by mycobacterial infections (*Mycobacterium tuberculosis* Complex). Considering previous studies on the dynamics of biocultural interactions which take into account information related from contextual associations and chronology, we can conclude that a tuberculosis-like disease was present in prehistoric populations from NW Argentina. Copyright © 2011 John Wiley & Sons, Ltd.

Key words: bone lesions; differential diagnosis; *Mycobacterium tuberculosis* Complex; NW Argentina

Introduction

Human tuberculosis (TB) is an infection caused mainly by *Mycobacterium tuberculosis* or *M. bovis*, mycobacteria which are members of the '*M. tuberculosis* Complex' that preferably target the respiratory tract. These bacteria may also cause gastrointestinal infection when contaminated products of animal origin are consumed (Zimmerman & Kelley, 1982; Ortner, 2003; Roberts & Buikstra, 2003). Tuberculosis is a disease that may present either acute or chronic characteristics (Aufderheide & Rodríguez-Martín, 1998). It is generally a biphasic disease (a primary infection phase followed by reinfection and/or reactivation phase). It is during the second phase when the infection may reach most body organs and tissues, even the skeleton (Powell, 1992; Aufderheide & Rodríguez-Martín, 1998; Ortner, 2003).

In the preantibiotic era, the incidence of skeletal TB averaged 5–7% of the patients, most of them being children (Steinbock, 1976; Zimmerman & Kelley, 1982; Aufderheide & Rodríguez-Martín, 1998). Clinical records indicate that skeletal involvement affects at least 1% of the diseased people (Davidson & Horowitz, 1970; Zimmerman & Kelley, 1982). However, Davies *et al.* (1984) studied patients with TB from England and Wales and found that 5% of them had developed osseous lesions. Thus, it is possible to infer that the prevalence of skeletal tuberculosis is not uniform across all modern populations, and its expression could be strongly related to the effects of nutrition and stress on the immune function, among other factors (Roberts & Buikstra, 2003; Wilbur *et al.*, 2008).

According to Aufderheide & Rodríguez-Martín (1998), the tuberculous disease is characterised predominantly by a destructive activity determining a pattern of lytic lesions (osteolysis) with little evidence of marked osteoblastic activity generating sclerosis and osteophytes around the lesions. Most of the tuberculous skeletal lesions involve the spine, as vertebral

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bodies are irrigated by arterial blood, which is rich in oxygen; this is a first-order condition for the bacilli of TB to proliferate. The involvement of posterior elements of vertebrae is very uncommon (Aufderheide & Rodríguez-Martín, 1998; Waldron, 2009). Vertebral lesions are mostly localised in the lower spine, in the inferior part of the thoracic segment and the lumbar spine (Allison *et al.*, 1981; El-Najjar, 1981; Resnick & Niwayama, 1989; Ortner, 2003; Roberts & Buikstra, 2003; Waldron, 2009). Cancellous bone of vertebral bodies may be destroyed up to a point in which such spongy tissue no longer resists and then collapses. The collapse of vertebral bodies (generally two or more vertebrae) not only causes a shortening of the trunk, but also a characteristic bending of the spine, kyphosis of Pott's disease (Zimmerman & Kelley, 1982; Aufderheide & Rodríguez-Martín, 1998; Ortner, 2003; Waldron, 2009). Pott's disease accounts for up to 50% of cases, generally being located at the lower level of the spine, from T8 to L4 (Allison *et al.*, 1981; El-Najjar, 1981; Resnick & Niwayama, 1989; Wilbur *et al.*, 2009). Rib and spine lesions are the most common lesions in TB. Around 9% of individuals with pulmonary tuberculosis also present lesions in the ribs (Kelley & Micozzi, 1984). Such lesions often consist of periosteal new bone formation (PNBF) localised in the inner side of the ribs. In addition, studies carried out on individuals from the Coimbra Identified Skeletal Collection (CISC) that suffered from TB during the preantibiotic era showed a frequency of periosteal manifestations on the visceral side of the ribs that oscillated from 85.7% to 90.9% (Santos & Roberts, 2001, 2006). Tuberculous lesions in long bones are considered rare or unusual. However, Santos & Roberts (2001) noted that 44.4% of the individuals from the CISC diagnosed with TB displayed periosteal reactions in long bones.

Around 90% of skeletal lesions also affect some articulations (Aufderheide & Rodríguez-Martín, 1998). Articular TB frequently produces a combination of osteomyelitis and arthritis (Messner, 1987). The articulations most commonly affected are those of the hip and knee (El-Najjar, 1981; Zimmerman & Kelley, 1982; Aufderheide & Rodríguez-Martín, 1998), followed by those of the elbow, wrist and shoulder (Aufderheide & Rodríguez-Martín, 1998).

Tuberculosis in South America before Columbus: presence, dispersion, and reactivation

For years, the acceptance of the occurrence of osseous manifestations indicative of TB-like infection in

pre-Columbian times remained a controversial issue (Buikstra, 1981; Aufderheide & Rodríguez-Martín, 1998; Ortner, 2003; Roberts & Buikstra, 2003). One of the strongest arguments against the hypothesis that supports the presence of pre-Columbian TB in the Americas was the extreme susceptibility of Native Americans to TB. This extreme susceptibility to the pathogen was considered as evidence that Native Americans reacted as an immunologically virgin population to the pathogen after their first contact with Europeans (Steinbock, 1976; Clark *et al.*, 1987; Dobyns, 1992). Currently, the presence of pre-Columbian TB in the Americas is a widely accepted fact.

Among the most noticeable studies about the presence of pre-Columbian TB in South America is the research by Allison *et al.* (1973), who worked on a mummified boy from the Nazca culture of southern Peru (AD 700) and performed the first confirmation of the presence of pre-Hispanic TB in South America. The mummy showed radiologic evidence of long-standing bone and soft tissue disease, and, more noticeably, the presence of acid-alcohol-resistant bacilli. Following the successful microscopic demonstration of the presence of mycobacteria in bioarchaeological remains, more pre-Columbian materials were taken into account, and the effective diagnosis of new cases of TB was then possible on a total of 11 mummies from Chile and Peru (Allison *et al.*, 1981). Later, Buikstra & Williams (1991) found 37 cases of TB among the skeletal series of Estuquiña, Southern Peru, dated approximately AD 1350. The strictly osteopathologic diagnosis was additionally confirmed by Salo *et al.* (1994), who published the first molecular diagnosis based on polymerase chain reaction (PCR) techniques on the aDNA of mycobacteria taken from a lung of a spontaneously mummified body of an adult female from the Chiribaya culture (AD 1000–1300, southern Peru). The same technique was successfully used on a mummified body of an 11–13-year-old girl from northern Chile, dated *ca.* AD 1040 (Arriaza *et al.*, 1995), and on skeletal remains of an adult male from the north coast of Peru, dated between 900 and 1100 AD (Klaus *et al.*, 2010).

In Argentina, a possible precedent about the presence of pre-Columbian TB among Patagonian hunter-gatherers is the finding of an individual at the Salitroso Lake basin (Santa Cruz) dated 728 \pm 39 years BP (Goñi *et al.*, 2003–2005; García, 2006). However, no previous studies have reported the finding of bony evidence for the presence of a TB-like disease among pre-Columbian populations in NW Argentina.

Among other aspects, the pattern of epidemiological behavior of TB is strongly bound to the presence of predisposing factors (conditions of general stress,

overcrowding, unhealthiness, malnutrition, etc.). The dynamics of prehistoric populations with economic basis on agricultural pastoralism in NW Argentina was accompanied by a series of sociocultural transformations that, according to archaeological evidence, derived into increasing processes of agglomeration of settlements at both local and regional levels (Albeck, 1992; Nielsen, 1997; Tarragó, 2000). On this scenario, it is possible to consider the existence of situations of marked stress either due to competition and defense of resources considered limited, or due to sustained attempts of territorial and sociocultural penetration that characterised the imperial expansion of the Incas. This paper aims to contribute new evidence on the existence of pre-Columbian TB in the Americas. It presents the first osteopathological data in the presence of TB among prehistoric populations from NW Argentina. It also suggests that in the case of the Late Ceramic Period societies in Santa María Valley, and well before the arrival of the Spaniards to the region, a previous imperial penetration – the expansion of the Incas – might have determined potentially disturbing, if not frankly disruptive, events. These situations could have favoured either the spread or at least the reactivation of a pre-Columbian illness such as tuberculosis.

Materials and methods

Rincón Chico 21 cemetery

Rincón Chico 21 (RCh21) is the name of a cemetery located in the extensive archaeological locality of Rincón Chico (Santa María Valley, Catamarca province). This locality, extending over an area of approximately 5 km², is located on the left margin of the Santa María River, approximately 5 km west of the city of Santa María (Figure 1), at the foot of the Eastern slopes of the Sierra de Quilmes or Cajón. The cemetery is partially segregated from any other archaeological indicator of human occupation (Mendonça *et al.*, 2005; Tarragó *et al.*, 2006; Croatto, 2008). The importance of this cemetery derives from indicators of diachronic as well as spatial complexity, and from the fact that in some sectors, particular conditions have favoured the preservation of organic materials. Funerary diversity includes direct primary inhumations, burial of children inside Santamaría tricolor style vessels, collective burials inside funerary chambers, as well as possible interments of small-sized ceramic offerings. Funerary chambers, or 'cists', are very well constructed by subvertical disposition of large flat stones enclosing a circular funerary reservoir (Tarragó *et al.*, 2006; Croatto, 2008).

In this study, we analysed a series of 70 skeletons from nine different collective burials systematically recovered during more than a decade of excavations at RCh21. Although the cemetery was partially looted in modern times, all the burials here considered were found intact. The contextual associations of these intact burials indicate that they are culturally and chronologically linked to the Period of Regional Developments (AD 850–1400), as well as to the early initial phases of the Inca Period.

Skeletal remains exhibiting the pathologies here considered were found buried inside chambers C-1, C-8 and C-11 D, as well as in Direct Interment II (ED II). The pathological sample consisted of six individuals. The skeletons were carefully excavated and recovered in such a way that they presented good preservation and integrity, with minimal postdepositional erosion of bone surfaces. All the bones were carefully treated and handled during excavation and recovery. Laboratory analyses included macro as well as microscopic observations, particularly of the spongy bony structure, of compact bone, as well as of proliferative bone responses. Radiological analyses were also performed in order to discard the presence of post deposit modifications (Micozzi, 1991; Aufderheide & Rodríguez-Martín, 1998; Ortner, 2003). Age estimation and sex determination were performed on pelvic and skull structures, as well as on metric and morphologic dimensions of several other postcranial elements (Buikstra & Ubelaker, 1994, and references therein). Systematic observations of traits and lesions were performed according to the *Standards for Data Collection from Human Skeletal Remains* (Buikstra & Ubelaker, 1994). Different methods and techniques for pathological recognition were applied according to the recommendations of different authors (Buikstra & Ubelaker, 1994, and references therein). The methodology employed during this research followed the steps below:

Determination of bone pathologies

The osteopathological conditions in each of the bone elements per skeletal individual were determined by simple ocular inspection, using a magnifier with artificial light, as well as a stereomicroscope Karl Zeiss, Stemi 2000-C model. Additionally, radiographs of every single pathological element were taken. In all the cases analysed, the criteria employed for identification, description, and osteopathological analyses followed the recommendations and indications published in the literature (Davidson & Horowitz, 1970; Steinbock, 1976; El-Najjar, 1981; Zimmerman & Kelley, 1982; Aufderheide & Rodríguez-Martín, 1998; Ortner, 2003; Santos & Roberts, 2001, 2006; among others). This

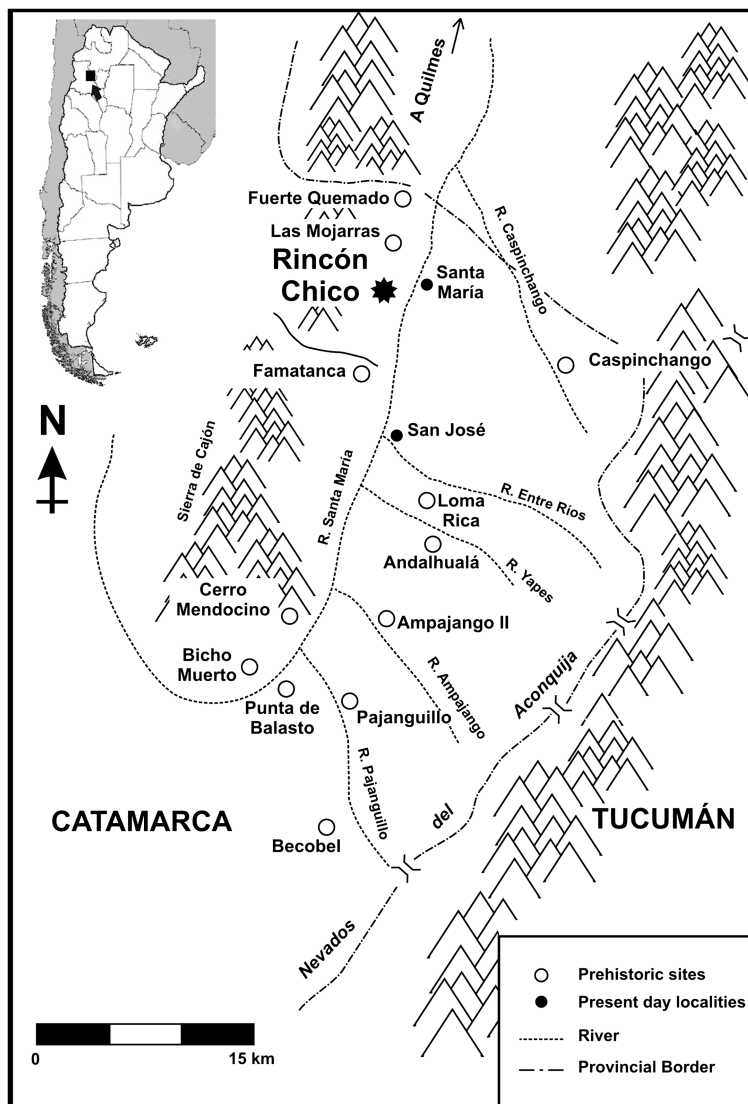


Figure 1. Valley of Santa María, or Southern Yocavil, Catamarca province. The asterisk in bold shows the position of the archaeological locality of Rincón Chico (modified from González and Tarragó, 2005).

information was used to perform a differential diagnosis on the six individuals displaying bone pathologies.

Chronologic determinations

Recovered materials at Rincón Chico 21 cemetery provided chronologic estimates of both absolute and relative dating. Six samples for radiocarbon dating were sent to Beta Analytic Inc. (Florida, USA): four human bone samples (to be dated by accelerator mass spectrometry (AMS), which is a technique that allows obtain radiocarbon dates from samples that are far tinier than that needed for standard radiocarbon dating), a piece of wood and a piece of charcoal (for conventional radiometry). Relative dating

is based on the existence of horizontal as well as vertical variations in the spatial distribution of features (i.e. simple direct interments, babies inside funerary vessels of Santamaría tricolor style, collective burials inside funerary chambers, stylistic characteristics of the funerary inclusions, clear stratigraphic intrachamber indicators of use and reuse of the same mortuary space, vertical distribution of funerary features indicative of constructive and interment sequences, and others) (Croatto, 2008; Mendonça *et al.*, in press).

Results

Six out of the 70 individuals analysed present bony lesions compatible with pathologies of infectious

origin. All the individuals belong to the Regional Developments Period (AD 850 to AD 1400). Table 1 synthesises the pathologies observed in the osteopathological assemblage analysed. A detailed description of these lesions for each individual is presented below:

Case 1 (C1-E#31). This case came from the first burial at the base of chamber C-1, which consisted of a burial of at least 25 individuals. The skeletal set had been intentionally displaced to make place for the last individual buried inside the chamber. There was a clear stratigraphy separating these two burial events. From the first multiple burial, it was possible to recover a partial skeleton consisting of several fragmented ribs and a segment of the spine (C3-T6). All these bones corresponded to the same adult individual, according to spatial and articular relationships, as well as matching techniques. Sex could not be established. Presence of PNBf was registered in the visceral surface of three left and two right rib fragments, and one without lateral identification. PNBf at the neck and the body of the rib fragments appeared in lamellar as well as woven form (Figure 2). A small lytic lesion was registered close to the upper edge of the 12th left rib. Irregular erosive lesions were observed in the head and neck at the vertebral end of a right costal fragment. As for the vertebral bodies, there was a clear example of well-healed vertebral lesions with kyphosis. The vertebral bodies of C7 and T1 presented massive destruction and had subsequently collapsed. The seventh cervical vertebra had lost a large part of its body mass, mainly in the infero-anterior portion. The body of C7 had collapsed and had fused with the superior surface of the body of T1, provoking a clear angular kyphosis (Figure 3).

Despite the kyphosis, the spinal canal remained unobstructed. The first thoracic vertebra also had lost a large part of its body mass, collapsing and then noticeably decreasing in height. The superior and inferior surfaces of the body of T1 remained intact, which suggested that the lesion had begun to develop inside the vertebral body. An irregular condensation of the trabecular bone of the bodies of C7 and T1 was observed in the saggital view of the radiological image (Figure 3B). Finally, as a consequence of the collapse of vertebral bodies, the apophyseal joints that articulated C7 - T1, and T1 - T2, were fused.

Case 2 (C8-E#B). Female adolescent (16.5–18.5 years old) from burial chamber C-8. The skeleton was recovered almost complete (only some bones from the hands and feet were missing). PNBf were registered in the visceral aspect of the neck of the fifth to seventh left ribs, and the fifth to eighth right ribs. Periosteal changes in ribs appeared in its lamellar type along with porosity, resembling elevated plaques. Moreover, the vertebral end of the fourth right rib presented a deep lytic lesion. Periosteal reactions were also observed in the anterior aspect of the distal metaphysis of the right humerus; the anterior aspect of the distal metaphysis of the left radius; behind the radial notch of the right ulna; the ilia of both os coxae; the proximal metaphysis and epiphysis of both femurs (the articulations themselves were not affected by PNBf, just the bone surfaces lined with periosteal tissue around them); the anterior aspect of diaphyses of both tibiae; the medial and distal third of both fibulae (Figure 4); and the fifth left metatarsal. The anterior face of the vertebral body of C4 presented an oval cavitating lesion of 0.4 × 0.5 cm, which had heterogeneous and porous walls, as well as an irregular bottom. The surfaces

Table 1. Distribution of the lesions by case, funerary structure, chronology, age and sex

Case	Feature	Period	Sex ⁽¹⁾	Age (yrs)	Lesions ⁽²⁾
1	C-1	Regional Developments-Inca	I	I	Destruction and collapse of vertebral bodies of C7 and T1. PNBf and lytic processes in ribs.
2	C-8	Regional Developments	F	16.5–18.5	Resorptive lesions in vertebral bodies (cervical, thoracic and lumbar). Systemic PNBf (ribs, right humerus, left radius, right ulna; both os coxae, femurs, tibiae and fibulae; and fifth left metatarsal)
3	C-11	Regional Developments	F	45–49	Pott's disease, erosive processes at left os coxa and PNBf at os coxae, tibiae, fibulae, right femur and left ulna.
4	C-11	Regional Developments	M	39–44	Lytic lesions in vertebral bodies of C7, T1, T2, T3, T8, T10 and T11.
5	C-11	Regional Developments	M	18–19	Systemic PNBf, marked in ribs, right os coxa and anterior (visceral) aspect of sacrum.
6	ED II	Regional Developments	F	13.5–15.5	[Destructive lesions in vertebral bodies of T10 and T11]. Multiple lytic processes at sternum. PNBf at ribs and right humerus.

⁽¹⁾F: Female; M: Male; I: Indeterminate.

⁽²⁾PNBf: Periosteal new bone formation.



Figure 2. Periosteal proliferation at the visceral surface of two ribs (case 1). A: Woven bone (disordered, irregular appearance, intense porosity). B: Lamellar bone (more homogeneous appearance, more density and less porosity).

of the anterior aspect of all dorsal vertebral bodies were pathologically eroded, with extension towards the right side. The trabecular bone that constituted the walls of the lesions had a heterogeneous and irregular appearance; no evidence of bone repair was observed. In T8

and T12, there were two irregular cavitating lesions with heterogeneous and porous walls that included approximately two thirds of the volume of the vertebral bodies. The body of T12 presented three deep lytic cavities on the anterior and left aspects, as well as on the inferior surface. The inferior cavity communicated with only one large, multilobulated, cavitating lesion. In the first lumbar (L1), there was a multilobulated cavity that included the whole upper and inferior surfaces of the vertebral body. The major portion of the inferior body of L1 was destroyed by a pathological process provoking a decrease in body height and determining the occurrence of kyphosis (Figure 5). The superior articular surface of L2 presented an irregular cavity extending deeply through the whole vertebral body. The surface of the lesions in T12, L1 and L2 was mainly constituted by heterogeneous and porous trabecular bone showing very little thickening.

Case 3 (C11-E#C). Adult female individual (45–49 years) from funerary chamber C-11. Although the skeleton presented a good degree of completeness, the first three thoracic vertebrae, all the lumbar vertebrae, several ribs, both clavicles, the left humerus and patellae were missing. Pronounced PNBf was present in the right ilium, in the left iliac fossa, in the anterior aspect of the right femur diaphysis, as well as in the distal third of the diaphyses of both tibiae. The fibulae and the left ulna also presented moderate PNBf. The periosteal changes were mostly of the woven type, with irregular, heterogeneous, unstructured and detachable appearance. Both sides of the left ilium had an abnormal irregular appearance due to erosive lesions surrounded by lamellar PNBf (Figure 6), which were observed at supra acetabular level and at the iliac fossa.

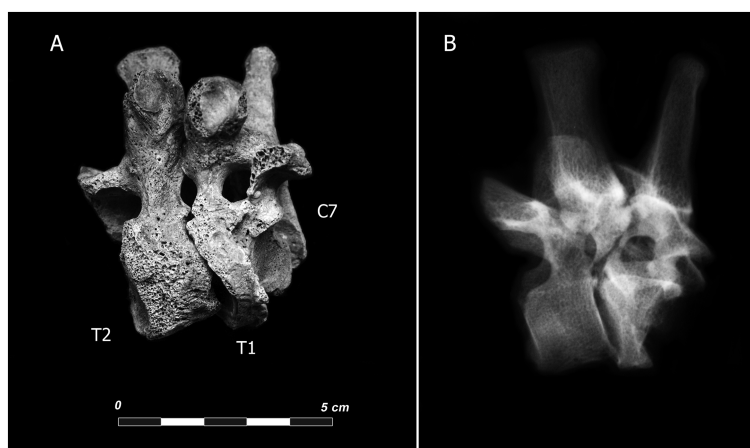


Figure 3. Lateral view (right) of three vertebral bodies C7, T1 and T2 (case 1). C7 and T1 are affected by lytic lesions. A: Collapse of C7 and fusion to T1 provoking kyphosis. The body of T2 presents post deposit modifications. B: Sagittal X-ray view of C7-T2 showing collapse of C7, loss in body height of T1, irregular condensation of the trabecular bone, fusion of vertebral bodies of C7 and T1 as well as the apophyseal joints in the three vertebrae.



Figure 4. Lamellar PNB (detail). Lateral aspect of the medial third of diaphysis from a left fibula (case 2).

The radiological image (Figure 6B) showed that in the site where the erosive lesion was present, there was a decrease in bone density, indicating the loss of spongy bone. The 12th dorsal vertebra (T12) had lost most of its body mass as a result of a lytic lesion. A noticeable cavity was observed in the central and inferior portions of the vertebral body, showing smooth edges and porous walls. The inner trabeculae were thickened and partially dissolved. The contiguous vertebra (T11) also presented a lytic lesion, but it was not analysed as it disintegrated during excavation.

Case 4 (C11-E#E). Adult male (39–44 years old) from burial chamber C-11. Most of the skeleton was recovered, with the exception of the skull, the atlas and some hand bones. In this individual, a cavity of 0.9 cm in diameter was observed at the anterior aspect of C7 vertebral body. The anterior aspect of T1 was pathologically eroded and presented a deep, oval, lytic lesion. The walls of the lesion were constituted by

heterogeneous and irregular bone. In T2 and T3, a noticeable multilobulated cavity involved the two vertebral bodies in such a way that the two pieces had lost more than half of their body mass. The above-mentioned cavity presented smooth edges and homogeneous walls, evidencing trabecular thickening. In T2, the cavity affected the whole anterior aspect of the body, crossing the entire body from anterior to posterior. The cavity measured 2.9 cm in width and 1.2 cm in height in the anterior aspect of the body, and also affected the inferior body surface. In T3, the cavity was 1.3 cm in diameter and 1.6 cm in depth, and affected the right side and the upper surface of the body (Figure 7). The eighth thoracic vertebra (T8) showed the formation of a lytic lesion of 0.6 cm in diameter in the anterior face of the vertebral body, whose irregular surface presented a woven aspect. The tenth thoracic vertebra (T10) presented a lytic lesion in the infero-anterior corner of the body. No evidence of proliferative responses was observed in the vertebral bodies affected.

Case 5 (C11-E#G). Adolescent male (18–19 years old) from funerary chamber C-11. The skeleton presented a very good degree of integrity and completeness, although the skull and the long bones of the arms, the fibulae and the patellae were missing. A PNB was observed in the internal face of the fragments of the fourth and fifth right ribs, one left and one non lateralised rib fragments. In the fourth right rib, PNB was located in the vertebral end. In the fifth right rib, PNB spread over a large part of the internal surface of the costal body. In the left rib, PNB was located in the internal aspect of the sternal end. In the remaining fragment, PNB was located in the internal surface of the costal body. In all the costal fragments, PNB was lamellar with little porosity. Periosteal reactions were also evident in the anterior

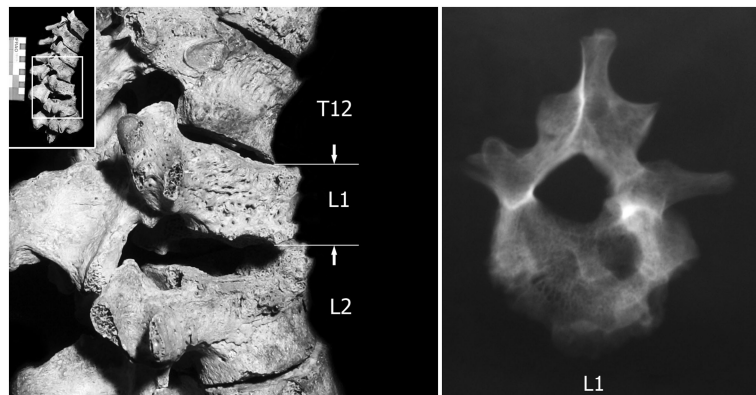


Figure 5. Lytic lesions at the spine (case 2). A: Lateral view (right) of a dorsolumbar segment of the spine (T11-L3) showing loss of body height of L1. B: X-ray (superior) view of L1. A radiolucent region that corresponds to a multilobulated cavity located inside the vertebral body.

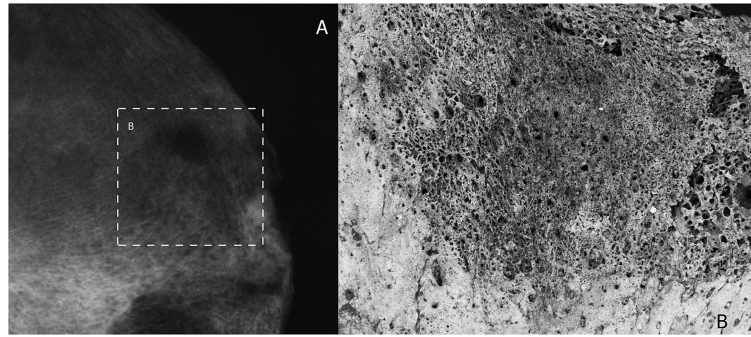


Figure 6. Erosive lesions at the ilium of the left coxa (case 3). A: X-ray showing a radiolucent region indicative of such lesions. B: Detail of the erosive lesion surrounded by lamellar PNBf at the insertion area for gluteus medius.

face of the vertebral bodies of S2, S3 and S4 of the sacrum, as well as on the whole iliac fossa and on the external supra acetabular table of the right os coxa. In the iliac fossa, PNBf is presented in lamellar type, as elevated plaques that are easily detachable.

Case 6 (EDII). Female adolescent (13.5–15.5 years old) from a direct interment. The skeleton could be recovered completely, although some of the bones presented *post mortem* modifications. In this individual, lamellar PNBf was observed in the visceral aspect of the neck of right ribs 9 and 10. The right humerus presented lamellar PNBf in the medial third of the diaphysis. (During the excavation process, it was observed that T10 and T11 displayed multiple lytic lesions in the anterior and lateral aspects of the vertebral bodies. However, the vertebral bodies with the observed pathologies also disintegrated during the excavation process). This individual also showed the presence of multiple lytic lesions in the visceral aspect of the manubrium as well as in the body of the sternum. These cavities had an oval form, whose diameter seldom exceeded 0.3 cm. Lesions in

the manubrium were concentrated in the center, sometimes coalescing (Figure 8). In the body, the lytic lesions were less numerous and smaller.

Discussion

Based on critical and detailed analyses of contemporary diseases that can provoke osseous changes partially or completely similar to those registered in this study, it is possible to formulate the considerations presented below.

Resorptive lytic foci constitute a predominant characteristic in the analysed individuals, which allows to rule out Scheuermann's disease, traumatic arthritis and spondyloarthritis. In the first case, lytic lesions present rectangular rather than rounded morphology, and kyphosis, when it occurs, is not angular. According to Rothschild *et al.* (1999), although the involvement of the anterior aspect of vertebral bodies also occurs in

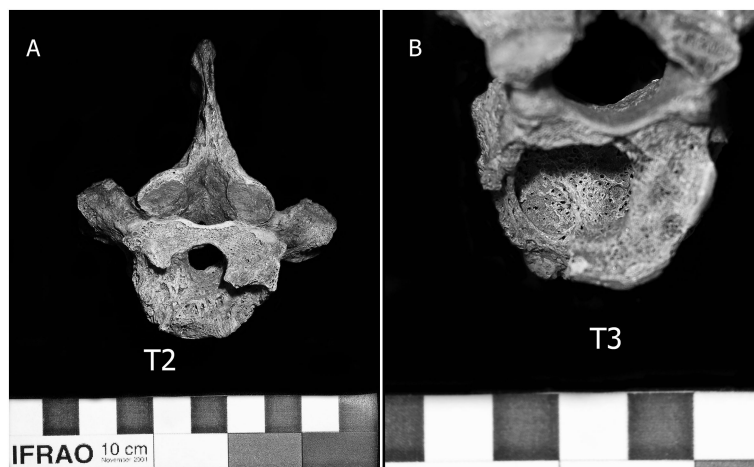


Figure 7. Lytic lesions in the bodies of T2 and T3 (case 4). A: Inferior view of T2 showing destruction of articular surface. B: Superior view of T3 showing a cavitating lesion intercommunicating with the T2 lesion. Both vertebrae show a thickening of the trabeculae at the lesion walls.

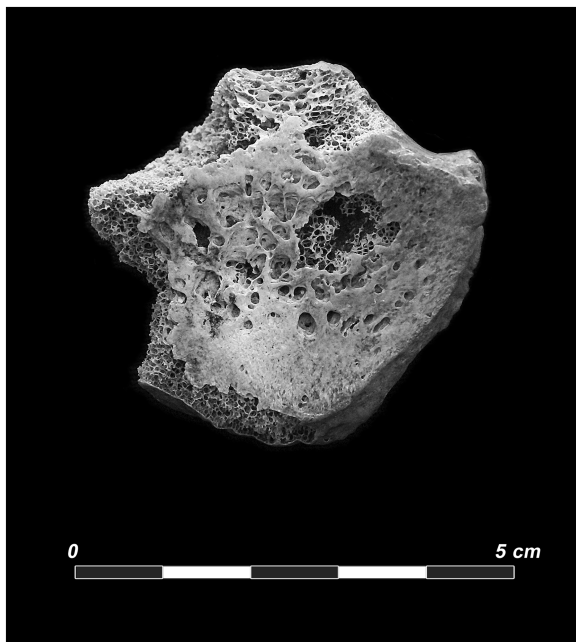


Figure 8. Lytic processes at visceral aspect of manubrium (case 6).

spondyloarthropathy, the rarefaction is much more common than cyst formation.

Additionally, a frequent, extensive vertebral involvement as observed in the bones here analysed is not a characteristic of healed vertebral fractures, septic arthritis, actinomycosis, chronic pyogenic osteomyelitis, coccidioidomycosis, blastomycosis or sarcoidosis. In the case of healed vertebral fractures, only one vertebra is often involved, and there is much less destruction of the vertebral body. In cases like these, kyphosis is not angular, and the presence of a great-ossified callus can be a distinctive trait (Ortner, 2003). Septic arthritis involves mainly the hip and knee joints (Ortner, 2003). It is a disease of rapid evolution, in which the articular involvement is often unilateral, but the osseous changes are comparatively less destructive (Aufderheide & Rodríguez-Martín, 1998). In actinomycosis, the osseous involvement is rare, being the mandible the most commonly affected area. At the vertebral level, it equally involves bodies and arches, while the intervertebral disc is not affected (Aufderheide & Rodríguez-Martín, 1998; Tayles & Buckley, 2004). Chronic pyogenic osteomyelitis is a disease of rapid evolution, accompanied by marked osteogenic processes where vertebral involvement is not frequent, affecting only one vertebra in 75% of cases (Ortner, 2003). Paravertebral formation of abscesses is rare, and there is formation of large sequestra (Reichs, 1989; Aufderheide & Rodríguez-Martín, 1998). Coccidioidomycosis as well as blastomycosis can determine the formation of resorptive lesions and an

extended vertebral involvement; the lesions, however, tend to be present in the bony prominences (e.g. acromion, tibial tuberosity, etc.) (Long & Merbs, 1981; Shadomy, 1981). Furthermore, when the disease attacks the spine, it also affects the posterior elements of the vertebrae, and no formation of a gibbus takes place (Long & Merbs, 1981; Aufderheide & Rodríguez-Martín, 1998; Ortner, 2003). Finally, sarcoidosis is a disease that can provoke multifocal lesions in vertebrae as well as in intervertebral discs, with occurrence of paraspinous masses (Resnick *et al.*, 1989). However, this is an extremely rare disease (Buikstra, 1976; Ortner, 2003; Tayles & Buckley, 2004) and does not present an extensive involvement of the vertebrae.

In the skeletons described here, a clear predominance of localised lesions in the thoracic region was detected. According to Buikstra (1976), this suggests that the typhoid spine would not be a potential causative agent. This disease is present in ribs, tibiae and also in lumbar vertebrae. Moreover, the vertebral bodies affected show reactive perifocal sclerosis, marginal body bridging and narrowing of the intervertebral disc (Aufderheide & Rodríguez-Martín, 1998).

Although echinococcosis may affect the skeleton, the skeletal involvement is not as common as that observed in the individuals analysed in the present study. Moreover, the lesions are well circumscribed, numerous and massive in scale (Klaus *et al.*, 2010). In the spine, although the disk spaces are not usually affected, the lamina and the adjacent ribs are generally involved (Aufderheide & Rodríguez-Martín, 1998). Additionally, transverse and spinous processes of the vertebrae are frequently affected (Ortner, 2003; Klaus *et al.*, 2010).

With regard to malignant bone tumors, carcinomas often manifest in mature or senile individuals, involving more than two non-contiguous vertebrae, as well as neural arches and ribs. When present, kyphosis is obtuse. A high rate of mortality among young adults is not expected for this illness (Buikstra, 1976; Klaus *et al.*, 2010).

Even though the osseous changes caused by brucella osteomyelitis can mimic those of TB, in brucellosis, the presence of osteoblastic remodeling of the lesions is considered pathognomic. This often results in a hemiring of greater density (osteophytes) around the lower part of the destroyed area, due to an increase of individual trabeculae in size and number in an attempt to repair the lesions (Capasso, 1999; Ortner, 2003; Tayles & Buckley, 2004). Although the spinal lesions locate in the vertebral bodies, specially of the lower thoracic, lumbar and lumbosacral areas, the most common site of the lesions is the supero-anterior margin of the vertebral body (Capasso, 1999; Ortner, 2003). In the

skeletons studied here, there was very little evidence of bone repair, and most lesions involved the center of the vertebral body, without affecting its antero-superior corner. Furthermore, brucella spondylitis occurs mainly in mature and senile individuals (Bodur *et al.*, 2004). Finally, brucellosis is considered an unlikely diagnostic option due to the involvement of contiguous vertebrae (Klaus *et al.*, 2010).

These considerations allowed us to evaluate the possibility of ruling out several diseases from a spectrum of possible diseases that may affect the skeletal system and could have mimicked the periosteal and lytic lesions present in the cases analysed here. Furthermore, it was possible to recognise, according to the distribution and characteristics of the lesions, the existence of a set of features that firmly signal a pattern related to the presence of a TB-like disease.

One remarkable feature is the predominance of lytic lesions affecting the spine in the skeletons studied. Moreover, most of the vertebral lesions were observed in the middle or inferior part of the thoracic region, as well as at the first lumbar vertebra, which is strongly related to what is presently known for the presence of a spinal infectious TB-like pattern (Allison *et al.*, 1981; El-Najjar, 1981; Resnick & Niwayama, 1989). Even though the registered lesions for Case 1 have a non-typical localisation, several researchers (Hartney, 1981; Widmer & Perzigian, 1981; Arriaza *et al.*, 1995; Aufderheide & Rodríguez-Martín, 1998:Table 7.2; Santos & Roberts, 2001; Tayles & Buckley, 2004) have described cases with similar distribution. In the individuals of the Cases 1, 2, 3 and 4, the presence of lytic lesions in vertebral bodies could be observed. These lesions are mostly localised in the anterior face of the vertebral body. This localisation is particularly clear in Case 2, where all thoracic vertebrae were affected. This could have resulted from the development of abscesses between the vertebral body and the anterior longitudinal ligament, a situation that might have favoured a caudal expansion of the infection (Aufderheide & Rodríguez-Martín, 1998). Other lytic foci are localised in the center of the vertebral body. These lesions provoked the destruction of most of the vertebral bodies, possibly as a consequence of successive expansions of an initial abscess in the inner part of the body. Finally, no signs of proliferative, reactive changes around the lesions were observed, and none of the vertebral lesions were localised in posterior elements of the vertebrae.

Regarding the periosteal reactions observed at the visceral surface of several ribs (four of the six cases), Kelley & Micozzi (1984) think that when found in a skeleton, this reaction is distinctive enough to predict its cause. These authors, as well as Davidson & Horowitz (1970), Eylar *et al.* (1996), Santos & Roberts (2001, 2006) and

Matos & Santos (2006), suggest that a PNBFB localised in the inner side of the ribs, in most of cases is associated with pulmonary TB. Nevertheless, there is the possibility that the pathology observed on the ribs may be due to a non-tuberculous pyogenic empyema. This pathology might occur when a phenomenon secondary to the extension towards pleural cavity of a case of pneumonia induces a periosteal response with similar characteristics in the rib. In this sense, as indicated by Aufderheide & Rodríguez-Martín (1998), it is necessary to consider that in the past, without the availability of the modern surgical drainage techniques, it is probable that very few individuals could have developed empyema from acute pneumonia. It is also unlikely that such individuals could have resisted long enough for such chronic periosteal reaction to take place. Capasso (1999) observed rib lesions in archaeological individuals suffering from brucellosis. However, the lesions are described as very small, isolated masses of PNBFB (button shaped), which are very different from the periosteal plaque that extends over the inner surface of the ribs reported in this study. From all the diseases taken into account here, Kelley & Micozzi (1984:386) consider that, in general, 'none of these conditions affect the ribs'. Even when the costovertebral reaction observed here might have developed from affected vertebrae, this explanation is not valid for the lesions observed in the ribs and sternum, because those lesions might have developed from a direct extension of a pulmonary lesion. Furthermore, in those cases where the identification of the affected rib was possible, the lesions were localised in the middle ribs. Similar results were obtained from the study of individuals with tuberculosis as a cause of death (Santos & Roberts, 2006). Noticeably, Cases 5 and 6 are the only ones that do not present lesions in the vertebrae (although we must keep in mind that, during the excavation process, in Case 6 we could observe that in fact, the bodies of T10 and T11 displayed multiple lytic lesions, but these vertebral bodies disintegrated). However, these Cases do present lesions in other bones. In these individuals, the ribs show PNBFB on the vertebral end, as well as on the costal body and on the sternal end. Although, as mentioned above, rib lesions are not exclusive of TB, it is possible to consider that their presence, localisation and distribution, combined with the co-occurrence of the rest of the pathologies discussed here, may be indicators that an infectious disease of respiratory origin was present among the individuals at Rincón Chico 21.

The origin of the lesion found in the sternum (Case 6) could be explained as a consequence of a direct extension of the infection from the lung due to: (i) the sternal

association with the other osseous indicators of lesions very possibly caused by an infectious pulmonary disease, and (ii) the high tension in blood oxygen in this region. Baxarias & Herrerín (2008) consider that, although not very frequently, this type of sternal lesion may be caused either by *M. tuberculosis* or by some atypical mycobacteria. Aufderheide & Rodríguez-Martín (1998) suggest that the localisation of lesions in flat bones such as ribs, os coxae, sternum and occasionally in flat bones of the skull vault could be a consequence of their hematopoietic function.

The lesions observed in the os coxae could be due to the diffusion of a disease by the closeness of affected organs. However, Aufderheide and Rodríguez-Martín (1998) consider that a vertebral abscess by *Mycobacterium* could produce a fistula in the anterior longitudinal ligament and expand the abscess towards caudal into the paravertebral muscles, thus affecting the psoas muscle. The psoas muscle fascia is dense enough to redirect the abscess until it reaches the tendinous insertion of the psoas at the lesser trochanter of the femur, thus producing lesions to the pelvis, especially in the groin or the iliac fossa itself. In the os coxae observed in this study, the lesions, both periosteal and lytic, developed mostly in the ilium, which, considering the distribution and localisation of the other osteopathologic lesions already mentioned for the rest of the skeleton, may well match with the origin mentioned by Aufderheide & Rodríguez-Martín (1998). With regard to periosteal changes found in the right os coxa of Case 5, considering the distribution of periosteal lesions described for the rest of the skeletal parts of this individual, it is also possible to infer that the origin of the lesion observed in that os coxa might be explained by hematogenous dissemination of the infectious agent.

Tuberculous lesions in long bones are considered rare or unusual. However, we need to keep in mind that in the metaphysis of those bones, there is spongy bone, richly supplied with arterial blood, which provides a potentially favourable environment for tubercle bacilli to prosper and in this way provoke a proliferative reaction of the periosteum as a response to a possible invasion of the pathogen. This may be the cause of the type of long bone lesions observed in this study. However, it is important to consider that proliferative periosteal

reactions can occur as a response to a large number of extrinsic and intrinsic pathological factors.

Chronological aspects

Absolute dating

Human bone samples (fragments of a tibia and three ribs) did not present collagen for AMS analyses, probably due to diagenetic alterations. Because of the absence of collagen, absolute datings were obtained from materials contextually associated to the burials (Table 2). These contextual datings place the materials analysed clearly inside the Late Ceramic Period and possibly at the initial stages of the Inca Period. Contextual associations and the excavation process are key factors. First, tombs C-8 and C-10, as all the tombs considered here, were found intact. Second, all ceramic inclusions in C-8 correspond to small bowls ('pucos') of Santamaría style, with good quality and decoration, characteristic of Regional Development Period (Marchegiani, 2008). Third, in C-10 (partially overlapping with C-11), the wooden shovel fragment sent for radiometric dating was found in a feature that is posterior to the construction of C-11. This funerary chamber can be considered from the Regional Developments Period, according to the funerary inclusions found in it. Additionally, the wooden shovel from C-10 was found inside a vessel of Santamaría tricolor style that was covered with an up-side-down bowl ('puco') of the same Santamaría tricolor style, both items being characteristic of the Regional Developments Period.

Contextual correlations

A remarkable aspect to consider is the location of the burials with individuals showing the pathologies analysed in this study. Their distribution strongly suggests spatial proximity and might correspond to the moment when either the dispersion or a reactivation of a pre-existing disease took place at the settlement. These circumstances lead us to consider the possibility that the process of the disease could have had epidemic characteristics. This possibility could be associated to conflict and/or social tension in a scenario of relationships of domination–resistance, such as those generated by the constant and sustained imperial penetration of the

Table 2. Dating of two contextually associated samples

Sample	Identification Code	Calibrated age	1 Sigma calibrated (68% probability)	2 Sigma calibrated (95% probability)
RCH21 C8CARBON	Beta-186150	Cal AD 1410	Cal AD 1320–1340 and Cal AD 1390–1420	Cal AD 1300–1440
RCH21 C10PAL	Beta-181724	Cal AD 1425	Cal AD 1405–1445	Cal AD 1310–1360 and Cal AD 1385–1480

Collasuyu in the Santa María Valley Regional Development settlements (Williams, 1994; González & Tarragó, 2005). This explanation is supported by the violent death of two individuals buried in the C-13 tomb, who had received at least 11 injuries with arrow points (Mendonça *et al.*, 2005).

Conclusions

The distribution of bone changes in the six skeletons studied strongly suggests a correspondence with the dynamics of a causative agent incorporated into the body via the pulmonary route, as well as a further hematogenous dissemination throughout the rest of the organism. The first observation is reinforced by the presence of periosteal manifestations in the ribs. Four out of the six individuals analysed present PNB in the ribs, particularly on the vertebral third of the visceral surface of the rib. The second observation is reinforced by the localisation of periosteal as well as lytic processes in the rest of the skeleton, which suggests the presence of a disease driven by miliary dissemination. Finally, the fact that at least four out of the six individuals studied display lytic processes in vertebral bodies, especially in the thoracic region and with no involvement of the arches, strongly suggests that these individuals suffered from a disease consistent with tuberculosis.

Although we decided not to include Cases 5 and 6 for the reasons mentioned above, the percentage of individuals affected by this disease (4/70) reaches 5.71% of the total of individuals assigned to the Regional Developments Period. This circumstance leads us to consider the relevance of performing careful systematic excavations in order to maximise recovery, observation and recording conditions, and the importance of our findings, considering the number of individuals affected and the intensity and endurance of the immune response, which indicates a long-lasting illness. According to the theory of the so-called 'osteological paradox' (Wood *et al.*, 1992), it is reasonable to evaluate the possibility that a larger number of individuals suffered from this disease, but died well before it could involve the skeleton. Therefore, the frail individuals never developed skeletal lesions because their immune system did not allow them to resist long enough to develop bone changes. In this scenario, it is probable that the evidence here considered points out the presence of a disease of epidemic magnitude, though immunologically not unknown. The presence of a mature male individual and a mature-senile female with TB-like lesions would be indicators of individual resistance even in conditions of extreme morbidity.

Radiometric dating on contextually associated materials (dating on wood and coal), as well as the relative dating related to spatial distribution, ceramic analyses and contextual associations, strongly support the idea that the observed phenomenon effectively corresponds to pre-Columbian times.

All these circumstances lead us to conclude that at Rincón Chico, an infectious TB-like disease, occurred in a period of time between Regional Developments and the effective imperial presence of the Incas in the Santa María Valley. On the basis of the currently confirmed existence of pre-Columbian TB in the Americas, and very particularly in the Andean region, it is possible to consider that Rincón Chico 21 cemetery corresponds to the existence of a pre-state society in NW Argentina where demographic density and poor health might have favoured a direct human-to-human transmission. In this scenario, and as a consequence of processes derived from an ever-increasing imperial penetration of the Incas, it is possible to consider a diffusion (or a reactivation) of a pre-existing disease as a probable interpretation.

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