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Hélène Coqueugnot, György Pálfi, Bernard Gély, Olivier Dutour

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1 **Title: UPPER PALEOLITHIC TUBERCULOSIS: A PROBABLE CASE ILLUSTRATED BY**
2 **PALEOIMAGING METHODS (AZILIAN SITE OF LES IBOUSSIERES, FRANCE)**

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5 **Author names and affiliations:**

6 Hélène COQUEUGNIOT ^{1,2,*}, György PALFI ³, Bernard GÉLY ⁴, Olivier DUTOUR ^{2,1}

7

8 ¹ UMR 5199 PACEA, Université de Bordeaux, Allée Geoffroy Saint-Hilaire, 33615 PESSAC cedex,
9 FRANCE

10 ² Ecole Pratique des Hautes Etudes, PSL Université Paris, 4-14 rue Ferrus 75015 PARIS, FRANCE

11 ³ Department of Anthropology, University of Szeged, Közép fasor 52, SZEGED 6726, HUNGARY.

12 ⁴ Ministère de la Culture DRAC Auvergne-Rhône-Alpes, Le Grenier d'abondance, 6 quai Saint-
13 Vincent, 69283 LYON cedex 01, FRANCE

14 * Corresponding author

15

16 **Corresponding author:** Hélène COQUEUGNIOT

17 e-mail: helene.coqueugniot@u-bordeaux.fr

18 Telephone: +00.33.6 98 44 40 35

19 **Present address:** UMR 5199 PACEA, Université de Bordeaux, Allée Geoffroy Saint-Hilaire,
20 33615 PESSAC cedex, FRANCE

21 **Abstract:**

22 The aim of this paper is to present the results of μ CT-scan and 3D imaging analyses of two
23 skeletal lesions observed on human remains of one of the last European hunter-gatherers from
24 the late Paleolithic (Azilian period): a sacroiliac osteoarthritis and a femoral lesion suggestive
25 of a soft tissue abscess imprint. These two skeletal elements (fused left sacrum and coxal bone,
26 and right femur) displayed osteometric criteria indicating that they belonged to the same
27 individual. These two associated lesions are consistent with a low-grade osteoarticular
28 infection, and suggest a diagnosis of pelvic tuberculosis with a cold abscess of the thigh. If
29 molecular confirmation is obtained, this case would be the first evidence of tuberculosis among
30 Upper Palaeolithic populations.

31 **Keywords:**

32 Tuberculosis; Hunter-gatherers; Upper Paleolithic; Azilian; Paleoimaging; 3D methods

33

34

35 **Text:**

36 1. Introduction

37 Tuberculosis is presented as one of the oldest human infections. Paleopathology can find its
38 traces in human remains from ancient times [1].

39 Although the Neolithic period was for a long time considered as the date of its emergence in
40 the human species and animal domestication its main cause [2], the phylogenetic study of the
41 group of bacteria responsible for human and animal tuberculosis (*M. tuberculosis* complex)
42 showed a very different evolutionary history, demonstrating an ancestry of the human-
43 adapted strain compared to the animal-adapted strains [3]. This result allowed authors to
44 estimate the emergence of the human-adapted TB strain in the early period of Paleolithic [4].
45 However, paleopathological research, whether morphological or molecular, has so far failed
46 to yield any convincing evidence of pre-Neolithic human tuberculosis [5]. Its earliest evidence
47 is dated to the emergence of the Neolithic in the Fertile Crescent, before domestication [6]. A
48 convincing case of tuberculosis infection in the Paleolithic has been presented, but it concerns
49 a North American Pleistocene bison [7]. To resolve this paradox, paleopathologists must focus
50 their attention on Paleolithic human remains in search of evidence of tuberculosis infection,
51 using morphological and/or molecular methods. Thanks to paleoimaging methods that
52 brought an added-value to retrospective diagnosis in paleopathology [8], we present here,
53 while awaiting molecular confirmation, a probable case of pelvic tuberculosis on one
54 individual from the last European hunter-gatherers population (final Paleolithic, Azilian
55 period, South-Eastern France).

56

57 2. Material and methods

58 2.1. Archaeological background

59 The site “Aven des Iboussières” is located near Malataverne, a small town of in South-Eastern
60 France near the city of Montélimar. The plain of Malataverne is overhung by an Urgonian
61 limestone massif, carved by an extensive karstic network, which is more than 600 meters long,
62 including several caves. This network is very unstable, making its exploration very dangerous
63 for speleologists. In 1990, speleologists explored the upper hall and discovered some human
64 remains. The instability of the network only allowed one rescue excavation, carried out in 1994
65 by Bernard Gély. Short time after this excavation, the collapse of the cavity vault prohibited
66 all further research. The east-west stratigraphic section distinguishes 5 layers (the fourth level

67 being itself subdivided into three sublevels A, B and C). The human remains and the great
68 majority of the archaeological and faunal material that have been collected, were coming from
69 this layer 4C. The ¹⁴C dates of animal and human bones from this layer provided similar results
70 dating this site at 10210±80 BP [9]. This site is exceptional due to the quantity and the diversity
71 of the objects of ornament and is considered as the richest in Western Europe [10]. This
72 material comes from layers 4C and 5 and is represented by ochre stones and engraved bone
73 pendants, perforated and decorated hedgehog hemi-mandibles, incised, engraved and ochre
74 bones, pierced teeth of deer, and marine shells.

75 2.2. Faunal diversity

76 Faunal remains are abundant and diversified [11]. Fourteen species were identified: Bison,
77 Auroch, Ibex, Chamois, Moose, Deer, Roe deer, Wild boar, Leopard, Lynx and smaller
78 mammals. Some of these remains presented marks of cutting, other were used as ornaments
79 such as anterior teeth of deer, perforated to be sewed on a garment, or tied like pearls for a
80 necklace, and small mammals' mandibles decorated with striations.

81 2.3. Human remains

82 The anthropological material was constituted by 291 bone fragments and 15 isolated teeth,
83 representing a minimum number of 9 individuals, 4 adults recognized as males and 5 children.
84 The general anthropological features are matched with those of Upper Paleolithic hunter-
85 gatherers including general robustness, dolichocrania, broad face, low and rectangular orbits,
86 platycnemia and high stature [12,13] (**figure 1**).

87 Paleogenomics analyses were performed on 3 individuals and showed that they belonged to
88 the U lineage of the N mitochondrial DNA haplogroup such as some of the more ancient
89 European hunter-gatherers (Dolni Vestonice) and the majority of the Holocene hunters-
90 gatherers [14].

91 2.4. Paleopathological observations

92 Among the pathologies previously noted [15], two skeletal lesions were reexamined that both
93 have an infectious pattern. The observation of two lesions of infectious origin on two different
94 bones in a small osteo-archaeological series (only 4 adult individuals), leads us to follow a
95 principle of parsimony and propose that these are two manifestations of the same infectious
96 process affecting the same individual. To support this hypothesis, we checked whether the
97 dimensions of the femoral head corresponded to the size of the acetabulum. These two bones

98 being opposed (left acetabulum and right femoral head), it was not possible to test the
99 anatomical congruence of the coxofemoral re-articulation directly, so we used an indirect
100 method by applying the formulae established by Plavcan et al [16]. These regression formulas
101 can be used to predict the diameter of an individual's femoral head from the size of his
102 acetabulum. Since there is no difference in size between the left and right femoral heads of
103 the same person [17], we can bypass the question of laterality here. By obtaining, from
104 acetabular size (diameter: 53.5mm), a calculated value of 47.175 mm for the femoral head
105 diameter, for a measured value of 47,4 mm, we can conclude that these two bones most
106 probably belong to the same individual. We have therefore re-examined these two
107 pathological bones from the same pathophysiological perspective, which enabled us to enrich
108 the diagnostic discussion.

109 The first lesion is a left sacro-iliac fusion which had been the subject of a first study by medical
110 tomography made in 1995, with, therefore, a resolution of low precision but which
111 nevertheless allowed to perform a first 3D reconstruction [18] and the second lesion, which
112 had not been analyzed by paleoimaging methods until now, which is present on the posterior
113 side of the right femur: it shows an irregular aspect, with periosteal reaction and polylobulated
114 imprints.

115 2.5. Paleoimaging methods

116 Microtomography acquisitions were performed using a GE v|tome|x S microtomograph. Coxal
117 bone was scanned at a voltage of 120 kV and a current of 200 μ A using a copper filter of
118 0.1mm. Images were acquired at a pixel size of 112 μ m.

119 Femoral diaphysis was scanned at a voltage of 100 kV and a current of 200 μ A using a copper
120 filter of 0.1mm. Images were acquired at a pixel size of 105 μ m.

121 The images were reconstructed with the TIVMI[®] v2.3 software based on the HMH (Half-
122 Maximum Height) algorithm [19] extended to 3D [20]. The segmentation of the inner
123 structures was processed manually by the first author (HC).

124

125 3. Results

126 As for left sacro-iliac fusion, the 3D reconstruction from μ CT slices revealed 2 distinct cysts,
127 one anterior and one posterior, overlapping the sacroiliac joint, having a mirror aspect, and
128 located on the iliac and sacral sides of the joint. The latter is partially fused (**figure 2**).

129 The posterior cyst is in direct communication with remnant of sacroiliac joint, constituting an
130 external pathway to lumbo-gluteal soft tissues (**figure 3**).

131 As for femur, it presents at middle shaft periosteal reactions and lobulated imprints of the
132 cortical surface (**figure 4**). The examination of the μ CT slices shows a typical aspect of cortical
133 osteitis under the lobulated imprints with adjacent periosteal reaction. It can be observed that
134 the medullar space and endosteum are strictly normal and that the periosteal reaction is not
135 circumferential but limited. This aspect of osteoperiostitis is very different from the one of
136 septic osteomyelitis which is characterized by invasion of the medullary zone and a
137 circumferential periosteal reaction.

138

139 4. Discussion

140 Diagnostic discussion focuses first on sacroiliac lesions. These are characterized by fusion of
141 the sacroiliac joint and the presence of two intraosseous cystic cavities. Unilateral ankylosis of
142 the sacroiliac joint can have three causes: degenerative, inflammatory or infectious.
143 Degenerative joint disease and DISH can lead to sacroiliac fusion, but are accompanied by
144 osteophytes with destruction of the joint in the first case, and thick ossification of the
145 sacroiliac ligaments without destruction of the joint space in the second; these two conditions
146 can be associated in elderly patients. Inflammatory spondyloarthropathies are very often
147 responsible for damage to the sacroiliac joint. The joint is affected, and fusion takes place
148 through fine, regular ossification of the sacroiliac ligaments. Age-related and inflammatory
149 causes can therefore be ruled out, and only infectious causes remain to be discussed.
150 Infectious sacroiliitis results either from pyogenic germs (*Staphylococcus aureus*,
151 *Streptococcus*, gram-negative bacilli), or from specific germs such as those of tuberculosis or
152 brucellosis. Pyogenic germs have a cellular toxicity that makes them highly destructive to bone
153 tissue, with a strong inflammatory reaction that increase bone lysis; chronicity is rare, and is
154 accompanied by bone sequestration and pronounced periosteal reactions; death was
155 frequent in the past in absence of treatment [21]. The aspect of the sacroiliac fusion is
156 therefore more suggestive here of a low-grade infection. Tuberculosis and brucellosis
157 infection have a granulomatous mode, the bone tissue reaction aimed at limiting the
158 progression of infection around the granulomas by peripheral sclerosis. Unlike brucellosis,
159 ankylosis is the natural history of sacroiliac tuberculosis [22]

160 As for cystic lesions, the discussion is more theoretical, as the main diseases responsible for
161 intraosseous cyst formation (aneurysmal bone cyst, unicameral bone cyst, cystic
162 angiomas) or bone tumours or dysplasias having a cystic appearance (non-ossifying
163 fibroma, fibrous dysplasia, giant cell tumor, chondroblastoma, chondromyxoid fibroma, or
164 even osteoid osteoma) show different localizations, aspect and are never responsible for joint
165 lesions and ankylosis. The association of intraosseous cystic lesions of the ilium and sacrum in
166 close contact with partially fused sacroiliac arthritis has no other explanation than an
167 infection. Brodie's abscess, corresponding to a subacute or chronic osteomyelitis with a "cyst
168 shape" may be discussed, as a very few cases were reported on posterior ilium in the literature
169 [23], some of them with gluteal syndrome, but sacroiliac joints were normal in these reported
170 cases. Brodie's abscess of ilium is presented as very unusual, on the contrary of cystic bone
171 lesions due to tuberculosis which are much more frequent, whatever the skeletal location:
172 spine [24]; distal epiphysis [25]; iliac bone near sacroiliac joint [26]. Moreover, tuberculosis is
173 known to produce mirror-image bone lesions on either side of an intervertebral disc,
174 metaphyseal plate or joint space [27], an aspect which is observed on our specimen, on the
175 two sides of the sacroiliac joint. This feature is not observed for Brodie's abscess. Therefore,
176 our conclusion is that the lesions we observed on this Azilian specimen (partial ankylosis of
177 sacroiliac joint with two osseous cysts overlapping the joint with a mirror aspect) is more in
178 favor of a tuberculous sacroiliitis than all the other discussed conditions.

179 Another element that adds to the discussion is the lesions observed on the posterior part of
180 the right femoral shaft. The first question is whether it could be septic osteomyelitis,
181 responsible for these irregular periosteal reactions. In this case, the infection starts in the
182 medullary spaces, crosses the cortical bone via the canaliculi and forms an abscess that
183 detaches the periosteum, stimulating osteogenesis and leading to the formation of an
184 involucrum. The analysis of μ CT slices excluded a possible diagnosis of septic osteomyelitis as
185 they showed an aspect of moderate osteoperiostitis which is very different (see results and
186 **figure 4**).

187 The interpretation is that the periosteal and cortical bone changes observed on this femoral
188 shaft are due to a slow-growing polylobulated mass located in the soft tissues of the posterior
189 thigh and in close contact with the bone. The moderate periosteal and cortical changes
190 observed on slices A to D (figure 4) are compatible with infectious abscess of low-grade, so
191 called "cold abscess".

192 To summarize, anthropological and paleopathological criteria converge to suggest that these
193 two bone elements belong to the same subject, and to advance a diagnosis of low-grade
194 infection, with sacroiliac osteoarthritis and cold abscess of the thigh. If this semiology was
195 observed on a today patient, the most probable diagnosis would be tuberculosis. The
196 sacroiliac joint is indeed a classic location for TB osteoarthritis, and cold abscess of the thigh
197 has been described in medical literature as a consequence of pelvic skeletal tuberculosis with
198 gluteal involvement [28–31]. We can assume that the cold abscess of the thigh results here
199 from a posterior migration of pus from the infected left sacroiliac joint under the gluteal
200 muscles, responsible for a flow of cold abscess from the sacral region to the posterior aspect
201 of the right thigh through the various intermuscular spaces of the gluteal muscles and the
202 lodges of thigh muscles, anterior and posterior. This led to the development of a cold abscess
203 in soft tissues of the middle part of the thigh. The abscess was in close contact with the
204 posterior part of the femur, developed in the space between the *fasciae* separating the
205 posterior muscle compartment from the anterior and medial compartments of the thigh
206 muscles (**figure 5**). The fact that the abscess was located on the right side of the thigh, whereas
207 the starting point was located on the left sacroiliac side of the thigh, was due to pain and
208 gravitational logic: the subject had to present a pain on the left side bothering him in a dorsal
209 or left lateral decubitus sleeping position. He had therefore to adopt an antalgic resting and
210 sleeping position on the right side, leading by gravity the passage of the tubercular pus from
211 the left side of the sacrum towards the spaces between the muscle lodges of the anterior and
212 posterior part of the right thigh.

213 To broaden the etiological discussion beyond the exclusive responsibility of *M. tuberculosis*,
214 the paleopathological lesions observed on these Paleolithic human remains are indicative of
215 skeletal infection by a low-grade pathogen, more likely a bacterial one. Best profile would be
216 a telluric bacillus, having a wild animal reservoir and granulomatous inflammatory mode
217 responsible for chronic lesions involving bones and joints. Ideally, it should have a triple way
218 of transmission (pulmonary, digestive and cutaneous). The bacteria having this profile belong
219 to the Actinobacteria phylum such as the genera *Mycobacterium* at the first place, followed
220 by *Nocardia* and *Rhodococcus*.

221 The archaeological context of the hunters-gatherers of the Iboussières site showed that they
222 were in close contact with a large spectrum of wild fauna, which are currently a natural
223 reservoir of these Actinobacteria, giving opportunity for microbial exchanges.

224 The aspect of the lesions is suggestive of a chronic stage of the infection, which is compatible
225 with a human-adapted infection, as are some of the strains of *Mycobacterium tuberculosis*
226 complex. This observation is in line with several phylogenetic models, according to which
227 human populations in Eurasia were infected with tubercle bacilli as early as the beginning of
228 the Upper Paleolithic [32]. The precise identification of the strain by paleomicrobiological
229 methods is in progress. These future results will be of great interest to understand more
230 precisely the evolution of human tuberculosis in Europe at the end of the Paleolithic.

231

232

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328

329

330 **Figure captions:**

331 Figure 1. Skull of male adult from the Iboussières site, facial view (A) and right lateral view (B).

332 Credits: O. Dutour

333 Figure 2. (A): 3D reconstruction of the sacrum and left ilium in superior view; (B): Transparency
334 of the 3D reconstruction of the two bones with superimposition of axial μ CT section. It reveals
335 two cysts: one anterior (red) and one posterior (green) overlapping the sacroiliac joint (dark
336 blue) which is partially fused; (C): Same axial μ CT section with the two cysts only. Credits: H.

337 Coqueugniot

338 Figure 3. Axial μ CT section showing the communication of the posterior cyst with the gluteal
339 space: (1) sacrum, (2) left ilium, (3) anterior remnant of the sacroiliac joint, (4) posterior cyst;
340 (5) communication of the posterior cyst through posterior remnant of the sacroiliac joint
341 towards the gluteal space (6). Credits: H. Coqueugniot

342 Figure 4. Portion of the right femoral shaft showing the level of μ CT slices from A to D. They
343 reveal a typical aspect of cortical osteitis (1) under the lobulated imprints (2) with adjacent
344 periosteal reaction (3). It can be observed that the medullar space and endosteum are strictly
345 normal and that the periosteal reaction is not circumferential but limited to the part
346 underlying the remodeled surface at the posterior face of femoral diaphysis. Credits: H.
347 Coqueugniot

348 Figure 5. Reconstructed section of the right thigh showing the development of the cold
349 abscess (1) in the space between the anterior (A) and posterior-medial (B) muscular lodges in
350 close contact with femur (2). Credits: O. Dutour

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