Paléorient

Prehistory of human tuberculosis: Earliest evidence from the onset of animal husbandry in the Near East

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Résumé
La tuberculose a été considérée, pendant longtemps, comme une zoonose transmise à l'homme par des bovins, notamment lors du processus de domestication de l'aurochs au Néolithique. Des travaux de phylogénie moléculaire récents ont remis en question ce dogme, montrant que le complexe Mycobacterium tuberculosis (MTBC) a existé comme pathogène humain depuis environ trois millions d'années. Cependant, des études récentes basées sur deux horloges moléculaires différentes ont proposé que la tuberculose humaine date de moins de 6 000 ans. Afin d'apporter de nouvelles données à ce débat, nous avons étudié les marqueurs paléopathologiques de la tuberculose sur des restes humains découverts dans le berceau proche-oriental de la Néolithisation, sur les sites de Dja'de el-Mughara (9310-8290 cal. BC) dans la moyenne Vallée de l'Euphrate (Syrie du Nord) et de Tell Aswad (8200-7500 cal. BC) au Levant central (Syrie du Sud). Ces deux sites ont livré chacun les restes squelettiques de plus d'une centaine d'individus qui ont fait l'objet de pratiques funéraires diverses. Les résultats obtenus par différentes approches (paléopathologie, micro-tomodensitométrie, paléomicrobiologie) confirment que ces vestiges constituent les plus anciens cas de la tuberculose humaine (un adulte et neuf immatures à Dja'de el-Mughara et un adulte à Tell Aswad) précédant et accompagnant l'émergence de la domestication des bovins au Proche-Orient. Parmi les onze cas identifiés, cinq individus ont été enterrés dans la Maison des Morts à Dja'de el-Mughara, les autres étaient inhumés dans des sépultures primaires, plurielles et mixtes. Sur la base de ces résultats, le futur défi serait de comprendre le rôle du contact étroit entre les humains et les animaux dans l'évolution du MTBC et les mécanismes d'émergence et de diffusion des souches modernes de la tuberculose humaine. Dans cette perspective, le Levant apparaît comme une région clé pendant les premières phases de la domestication animale et de la sédentarisation.

Abstract
Human tuberculosis has been considered for a long time as a model of animal infection transmitted to humans, resulting from cattle domestication at the Neolithic period. A decade ago, studies of molecular phylogeny of the Mycobacterium tuberculosis complex (MTBC) has challenged this dogma, suggesting that this human infection could be as old as the human species and emerged ca 2-3 Myrs ago. Yet, recent studies of molecular clock computations proposed that human tuberculosis could not be older than 6 kyrs BP. In order to bring new data to this debate, we studied the paleopathological evidence of tuberculosis on a large sample of two Neolithic sites from Syria in the Near East, cradle of agriculture and domestication : Dja'de el-Mughara (9310-8290 cal. BC) located in the Middle Euphrates Valley (Northern Syria) and Tell Aswad (8200-7500 cal. BC) in the Central Levant (Southern Syria). Both sites have delivered skeletal remains of more than one hundred individuals deriving from different funeral contexts. We used methods of paleopathology, microstructural analysis (µ-CT) and paleomicrobiology. The paleopathological study gave evidence to the most ancient paleopathological known cases of human TB (one adult and nine immature individuals at Dja'de el-Mughara and one adult at Tell Aswad) predating or accompanying the emergence of animal domestication. Among the eleven cases identified, five individuals from Dja'de el-Mughara have been buried in the House of the Dead, while the other individuals at both sites were found in primary, plural and mixed burials. On the basis of these results, the future challenge would be to understand the close contact between humans and animals role in the evolution of MTBC and the mechanisms of modern human tuberculosis strains emergence and spread. For this reason, the Levant is a crucial region as a key center for domestication and sedentism origins.

PREHISTORY OF HUMAN TUBERCULOSIS: EARLIEST EVIDENCE FROM THE ONSET OF ANIMAL HUSBANDRY IN THE NEAR EAST

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Résumé : *La tuberculose a été considérée, pendant longtemps, comme une zoonose transmise à l'homme par des bovins, notamment lors du processus de domestication de l'aurochs au Néolithique. Des travaux de phylogénie moléculaire récents ont remis en question ce dogme, montrant que le complexe* Mycobacterium tuberculosis *(MTBC) a existé comme pathogène humain depuis environ trois millions d'années. Cependant, des études récentes basées sur deux horloges moléculaires différentes ont proposé que la tuberculose humaine date de moins de 6 000 ans. Afin d'apporter de nouvelles données à ce débat, nous avons étudié les marqueurs paléopathologiques de la tuberculose sur des restes humains découverts dans le berceau proche-oriental de la Néolithisation, sur les sites de Dja'de el-Mughara (9310-8290 cal. BC) dans la moyenne Vallée de l'Euphrate (Syrie du Nord) et de Tell Aswad (8200-7500 cal. BC) au Levant central (Syrie du Sud). Ces deux sites ont livré chacun les restes squelettiques de plus d'une centaine d'individus qui ont fait l'objet de pratiques funéraires diverses. Les résultats obtenus par différentes approches (paléopathologie, micro-tomodensitométrie, paléomicrobiologie) confirment que ces vestiges constituent les plus anciens cas de la tuberculose humaine (un adulte et neuf immatures à Dja'de el-Mughara et un adulte à Tell Aswad) précédant et accompagnant l'émergence de la domestication des bovins au Proche-Orient. Parmi les onze cas identifiés, cinq individus ont été enterrés dans la Maison des Morts à Dja'de el-Mughara, les autres étaient inhumés dans des sépultures primaires, plurielles et mixtes. Sur la base de ces résultats, le futur défi serait de comprendre le rôle du contact étroit entre les humains et les animaux dans l'évolution du MTBC et les mécanismes d'émergence et de diffusion des souches modernes de la tuberculose humaine. Dans cette perspective, le Levant apparaît comme une région clé pendant les premières phases de la domestication animale et de la sédentarisation.*

Keywords: *Syria; Neolithic; Domestication; Early PPNB; Tuberculosis; Paleopathology.*
Mots-clés : *Syrie ; Néolithique ; Domestication ; PPNB ancien ; Tuberculose ; Paléopathologie.*

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INTRODUCTION

Lifestyles of human populations progressively and deeply changed during the Neolithization process, which spread over several thousand years in the Near East. Among other consequences, the advent of plant and animal domestication at the beginning of the Holocene (Diamond 1997; Peters *et al*. 2005; Zeder 2008; Willcox 2013) modified the equilibrium of the host-pathogen relationships and the pathocenoses, according to the concept developed by Mirko Grmek (1969),[1] and were responsible for the emergence of new human diseases due to bacterial species jump. Indeed, domestication process brought closer several animal species, including human, therefore possibly favoring the transmission of animal pathogens to human populations and vice versa—anthropozoonoses—(Woolhouse *et al*. 2005).

Tuberculosis was considered for a while as a model of anthropozoonosis, resulting from cattle domestication in the Neolithic period. Since 15 years, new phylogenies of different bacterial species involved in human tuberculous infection, referred as the *Mycobacterium tuberculosis* complex (MTBC), suggest that the common ancestor of the animal strains could already have been a human pathogen (Brosch *et al*. 2002). Then, genetic data help us to estimate that common ancestor of *M. tuberculosis* complex is as old as 3 million years, and human tubercle bacilli moved with *Homo sapiens* "out of Africa" following an "evolutionary bottle-neck" in the disease (Gutierrez *et al*. 2005). Some paleopathological elements on bison remains indicated that *M. tuberculosis* could have existed before the Neolithic (Rothschild *et al*. 2001). Recently, some authors contradicted this model, arguing that the origins of this human infection are not older than 6000 years BP (Bos *et al*. 2014).

Up to now, what is the earliest archaeological evidence of human tuberculosis and on what criteria is this based? The oldest case of tuberculosis would have been reported on a fragmentary skull of *Homo erectus* found at Kocabaş site in Turkey (Kappelman *et al*. 2008). Endocranial surface exhibited tiny changes that have been interpreted as possibly resulting from tuberculous leptomeningitis. However, the validity of this diagnosis was strongly refuted by other authors (Roberts *et al*. 2009) and this case was no more debated afterwards.

Other cases have been reported from two Pre-Pottery Neolithic sites showing evidence of agriculture and animal domestication, *e.g*., 'Ain Ghazal (El-Najjar *et al*. 1996) and Atlit Yam (Hershkovitz *et al*. 2008).

According to El Najjar *et al*. (1996), lesions strongly suggesting cases of tuberculosis were observed on cervical or thoracic vertebrae of three adult individuals uncovered in a multiple burial belonging to the Pre-Pottery Neolithic C levels at 'Ain Ghazal (6900-6400 cal. BC), near Amman, Jordan. Nevertheless, it seems that many differential diagnoses could explain the observed lesions (Hershkovitz and Gopher 1999; Roberts and Buikstra 2007) and further investigations are needed.

The site of Atlit Yam, which is now below sea level, is located close to the city of Haifa, Israel. It was occupied between 7300 and 6210 cal. BC, during the Pre-Pottery Neolithic C period (Galili *et al*. 2002). Paleopathological lesions, highly suggestive of tuberculosis, were observed on the remains of an adult female and of a child about 12 years old buried together. Ancient DNA analysis performed on the bones of these two individuals allowed the detection of the *Mycobacterium tuberculosis* complex. Mycolic acid lipid biomarkers, specific for the MTBC, were also detected (Hershkovitz *et al*. 2008). Until recently, this case was the oldest known.

In a previous paper (Baker *et al*. 2015), a pluridisciplinary set of analyses carried on the human bones found at two Pre-Pottery Neolithic sites in Syria, Dja'de el-Mughara and Tell Aswad, reported strong evidence of tuberculosis on individuals dating to earlier phases of the Neolithization process, corresponding to pre- and early animal domestication phases. In this paper, results from more recent analyses of additional material from these sites are presented along with more detailed information about the archaeological and funerary contexts.

CONTEXTS AND MATERIAL

DJA'DE EL-MUGHARA (9310-8290 CAL. BC)

The tell of Dja'de el-Mughara, located on the western bank of the Euphrates river in Northern Syria (figs. 1 and 2), has been excavated from 1991 to 2010 by a French team directed by one of us (EC). It concerns a crucial phase in the process of Neolithization and is the only known settlement in the Northern Levant covering most of the 9th millennium in a continuous sequence. Basal levels (phase DJ I, *ca* 9310-8830 cal. BC) correspond to the end of the Pre-Pottery Neolithic A (PPNA), as a transition phase to the Early Pre-Pottery Neolithic B (PPNB).

1. This concept is related to the dynamics of infectious diseases and their spatial and temporal evolution.

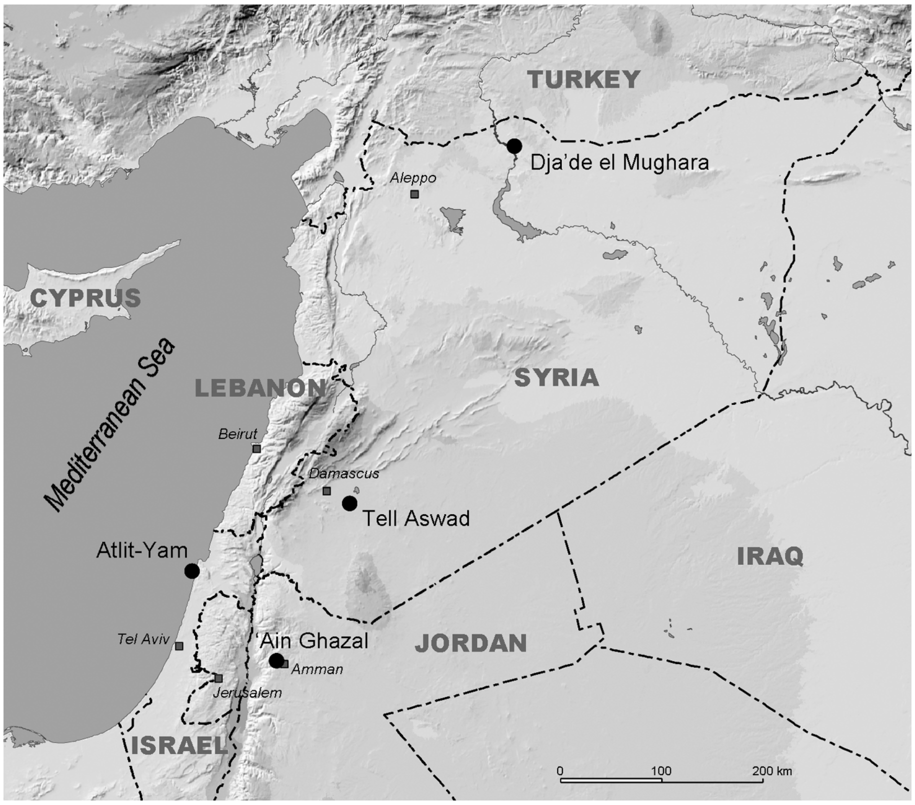


Fig. 1 – *Map of Syria showing the location of Dja'de el-Mughara and Tell Aswad (©Maison de l'Orient et de la Méditerranée, Lyon).*

Fig. 2 – *Dja'de el-Mughara.* ***1****) View of the excavation;* ***2****) close-up of a collective burial from the House of the Dead (DJ III).*

The following phase (DJ II, *ca* 8800-8500 cal. BC) corresponds to the beginning of the Early PPNB. Finally, phase DJ III (*ca* 8540-8290 cal. BC) fits the classical Early PPNB (Coqueugniot 1998; 2000 and 2016), and was later covered by a short Pre-Halaf settlement. Proto-agriculture was practiced since the first occupations (Willcox 2008) and the earliest evidence of the domestication of aurochs was found in the most recent PPN occupations (DJ III) (Helmer *et al.* 2005).

Many burials were discovered at this site and various mortuary practices could be evidenced (Chamel 2014). Human remains were recovered in all three phases but most of them belong to phase DJ III; they represent a minimum number of 130 individuals (Baker 2014; Chamel 2014) (table 1). Most of the skeletons were deposited inside a special rectangular stone building named "Maison des morts" ("House of the Dead") by the excavators (Coqueugniot 1998).

Table 1 – *Dja'de el-Mughara. Distribution of the human skeletal sample.*

Phase	Period	Immature individuals	Adult individuals	Total
DJ I 9310-8830 cal. BC	Late PPNA	1	4	**5**
DJ II 8800-8500 cal. BC	Early PPNB	8	18	**26**
DJ III 8540-8290 cal. BC	Early PPNB	60	39	**99**
Total		**69**	**61**	**130**

The skeletal remains from Dja'de el-Mughara were examined by two of us (OB and BC), in their integrity. In addition to at least 69 immatures, the whole adult sample comprises 9 males, 7 females and 45 undetermined individuals (table 1).

TELL ASWAD (8200-7500 CAL. BC)

This site, located in the Central Levant (figs. 1 and 3), has been excavated first by H. de Contenson (1995) in 1971 and 1972 and later by a Franco-Syrian archaeological mission conducted by one of us (DS) and B. Jammous from 2001 to 2007 (Stordeur *et al.* 2010). According to the revised stratigraphy and new radiocarbon dates (*Ibid.*), the occupation of the site can be divided into three PPNB phases: the first one (*Phase ancienne*) corresponds to the end of Early PPNB, the second phase (*Phase moyenne*) covers the Middle PPNB and the third one (*Phase récente*) dates from the end of Middle PPNB to the beginning of Late PPNB. Domestic animal and plant resources had been exploited since the Middle PPNB (Helmer and Gourichon 2008 and 2017; Stordeur *et al.* 2010). The site provided the human skeletal remains of at least 119 individuals (table 2). The funeral practices reveal the use of simple burials but also of multiple burials as well as evidence of skull removal. Furthermore, the results of the anthropological study (Khawam 2014 and 2015) indicate the presence of various treatments of the removed skulls, including plastered skulls, which correspond to ritual and funeral practices. During the earliest phases, the funerary deposits are found inside the houses, as part of a family unit. Later in the sequence, the corpses were buried in outdoor funeral areas. The spatial organization of these areas gives precise indications about the social order and the cultural identity of the Neolithic PPNB society at Tell Aswad (*Ibid.*).

Table 2 – *Tell Aswad. Distribution of the human skeletal sample.*

Phase	Period	Immature individuals	Adult individuals	Total	Examined adults by periods
Phase ancienne	End of Early PPNB	10	5	15	3
Phase moyenne	Middle PPNB	19	9	28	9
Phase récente	End of Middle PPNB - Late PPNB	27	49	76	28
Total		**56**	**63**	**119**	**40**

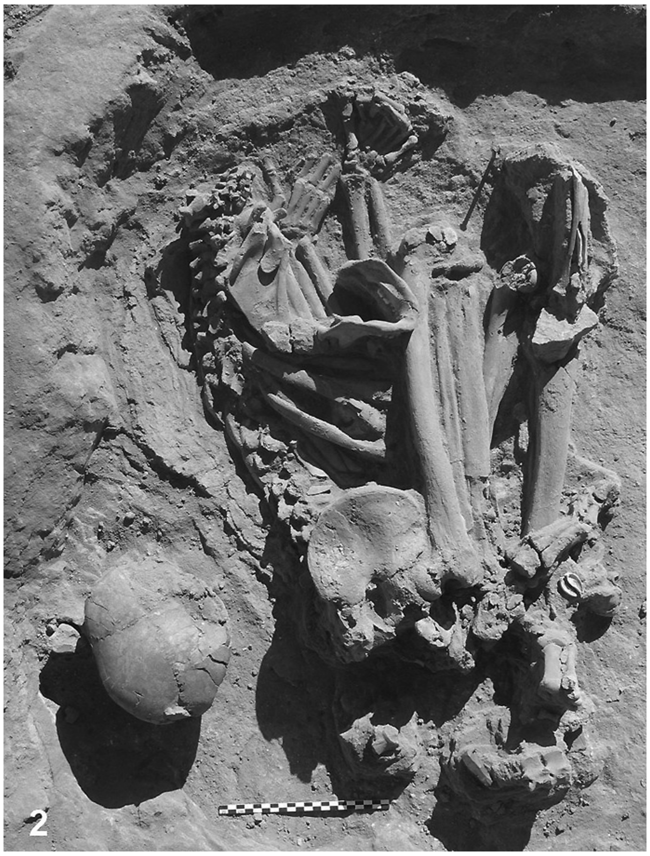
The studied human bone sample from Tell Aswad includes, due to the unforeseen political circumstances in the region, only the adults (9 males, 11 females and 20 undetermined individuals).

METHODS

The study of the assemblage was first performed in Syria at the archaeological storage places of Dja'de el-Mughara and Tell Aswad sites, and was continued in France afterwards, on a part of the material, in two research laboratories (Archéorient, CNRS, UMR 5133, Lyon; and PACEA, CNRS, UMR 5199, Bordeaux). A combined set of methods was applied, consisting of anthropological and paleopathological analyses, 3D scanning and molecular biology.

Sex and age-at-death estimations were carried out according to currently established methods (Moorrees *et al.* 1963a and b; Scheuer and Black 2000; Bruzek 2002; Murail *et*

Fig. 3 – *Tell Aswad. **1**) View of the excavation; **2**) young adult from burial 509 "a509-Aw".*



al. 2005; Schmitt 2005 and 2008; Coqueugniot *et al*. 2010), and different morphological criteria were applied for paleopathological diagnosis. Besides the classical expression of vertebral TB (spondylodiscitis also known as spinal destruction or Pott's disease) we scored less typical changes such as osteoarthritis, rib periostitis (periostal new bone formation on visceral surface of rib), *serpens endocrania symmetrica* (endocranial bone lesion due to meningitis TB; Hershkovitz *et al*. 2002), hypervascularization of the immature vertebral body (Baker 1999; Dutour 2008 and 2016) and periosteal changes attributed to hypertrophic osteoarthropathy (diffuse periosteal new bone formation) (Dutour 2011; Baker 2014; Baker *et al*. 2015).

We adopted two parameters for assessing the prevalence of TB: the crude and the corrected prevalence (Waldron 1994; Dutour 2008). The crude prevalence (P) is estimated by dividing the number of observed cases (n) of TB by the total number of skeletons (N1). However, we chose to exclude nine individuals from Dja'de el-Mughara and twelve individuals from Tell Aswad represented by scattered skulls and mandibles. The five individuals from the Late PPNA phase of Dja'de el-Mughara (DJ I) were not taken into account. We used this corrected number as a second dominator (N2) for calculating the crude prevalence of disease in the Early PPNB period, including 116 individuals for the first site (20 in DJ II and 96 in DJ III) and 28 for the second. The corrected prevalence is calculated per anatomical part and corresponds to the ratio number of TB cases / total number of anatomical elements present by considering unobservable bones (incomplete, fragmented and missing).

In order to analyze these exceptional specimens in the most optimal conditions and to protect them as scientific patrimonial heritage at the same time, digital imaging scanning was carried out on different available facilities in the research units of the University of Bordeaux, using laser surface scanning as well as µCT scanning (Coqueugniot *et al*. 2015).

Furthermore, synchrotron radiation-based tomography analyses were recently performed on a part of the sample, in the framework of a Synchrotron Soleil research project headed by one of us (HC). It focuses on the ultrastructural detection of early stages of TB infection on the skeletal material.

Actually, little is known about the first stages of tuberculosis infection on bones. They are responsible for inner microstructural changes and therefore are not directly visible on the bone. The understanding of the spread of infection through the cortical bone and its effects on the canal network are particularly relevant (Resnick and Niwayama 2002). Previous works (Coqueugniot *et al*. 2015; Baker *et al*. 2015) demonstrated the value of CT and µCT for the microstructural analysis of the early pathological processes on ancient and rare skeletal material, even when crucial elements of the cortical bone microstructure organization are not visible using µCT, namely the osteons lamellae and cement lines delimiting the osteon border. These criteria are really important to identify normal remodeling process of bone microstructure (Cooper *et al*. 2011; Maggiano *et al*. 2016) and therefore for recognizing early pathological changes.

In addition to these morphological microscopic analyses, two different molecular approaches were used to confirm the morphological diagnosis: the study of lipid biomarkers and ancient DNA (Baker *et al*. 2015).

In a first step, analysis of lipid biomarkers such as mycolates and mycocerosates was conducted at the Institute of Microbiology and Infection, School of Biosciences, University of Birmingham (Minnikin *et al*. 1993; Lee *et al*. 2012; Baker *et al*. 2015). Biomolecular analysis (amplification and sequencing) was performed to identify the specific insertion sequences IS6110, a molecular signature of MTBC infection (Donoghue *et al*. 1998; Haas *et al*. 2000; Zink *et al*. 2001; Baker *et al*. 2015) at the EURAC Institute for Mummies and the Iceman, Bolzano, Italy.

RESULTS

Among the osteoarchaeological sample representing a total of 170 individuals, 11 possible cases of TB infection (ten individuals for Dja'de el-Mughara and one for Tell Aswad) were identified on the basis of morphological criteria.

Five cases have been described previously (Baker *et al*. 2015): i483-Dj, i675-Dj, B108-Dj, a304-Dj and a509-Aw (table 3). We present here six new cases of TB from Dja'de el-Mughara: i278-Dj, C108-Dj, I108-Dj, J108-Dj, R108-Dj and i259-Dj (table 3).

Three of the ten cases observed at this site belong to the early phase of PPNB (DJ II, 8800-8500 cal. BC), where so far no evidence of ungulate domestication was found (Helmer *et al*. 2005). Seven other cases correspond to the more recent phase (DJ III, 8540-8290 cal. BC) where first stages of cattle domestication have been attested using biometric methods (Helmer *et al*. 2005). Tell Aswad has provided one case from the phase "*Aswad récent*" (end of Middle PPNB to Late PPNB).

Typical lesions were observed on two individuals (a304-Dj and B108-Dj), representing an early-stage spondylitis and a cystic aspect of spinal TB (table 3). Hypertrophic osteoarthropathy was observed in four cases (i483-Dj, i675-Dj, i278-Dj and a509-Aw), spina ventosa in one case (a509-Aw),

Table 3 – *Dja'de el-Mughara and Aswad. Summary of the paleopathological analysis of bone samples from 11 individuals.*

Period	Individual	Burial reference	Paleopathology	3D imaging and microCT	aDNA	Lipid biomarkers
DJ II (Early PPNB)	i483-Dj (1 yr)	483	SAVCh, LBP		PCR+ Baker *et al.* 2015	Positive profile Baker *et al.* 2015
	i675-Dj (8-10 yrs)	675	SAVCh, LBP, PR		PCR-	n.d.
	i278-Dj (4-5 yrs)	278	HOA, SAVCh		n.d.	n.d.
DJ III (Early PPNB)	a304Dj (adult)	304	ST	CVT	PCR-	n.d.
	C108-Dj (4-5 yrs)	108	SAVCh		n.d.	n.d.
	I108-Dj (6-13 yrs)	108	SAVCh		n.d.	n.d.
	J108-Dj (1 yr)	108	SAVCh		n.d.	n.d.
	R108-Dj (4-5 yrs)	108	SAVCh		n.d.	n.d.
	i259-Dj (12-18 yrs)	259	SAVCh		n.d.	n.d.
	B108-Dj (4-5 yrs)	108	SAVCh	ESS	n.d.	n.d.
"Aswad récent" Middle PPNB/ Late PPNB	a509-Aw (a.k.a. 509-H1) (20-22 yrs)	509	HOA	OM (ulna)	PCR-	Positive profile Baker *et al.* 2015

Paleopathological changes: SAVCh: superficial anterior vertebral changes; CVT: cystic vertebral tuberculosis; OM: osteomyelitis; LBP: long bone periostitis; ST: spinal tuberculosis; HOA: hypertrophic osteoarthropathy; PR: periostitis of rib; ESS: early stage of spondylitis
aDNA: (PCR +) positive result; (PCR -) negative result; (n.d.) not determined.

rib lesions in one case (i675-Dj), and vertebral hypervascularisation in nine immature cases (i483-Dj, i675-Dj, i278-Dj, B108-Dj, C108-Dj, I108-Dj, J108-Dj, R108-Dj and i259-Dj).

From the three cases belonging to the early pre-domestication DJ II phase, the immature i483-Dj is the only one to present a positive identification of the lipid biomarkers and molecular identification of *Mycobacterium tuberculosis* IS6110 specific sequence (Baker *et al*. 2015) (table 3).

The individual results are the followings:

DJA'DE EL-MUGHARA

– **i483-Dj individual** (DJ II phase), an infant *ca* 1 year old at death: superficial vertebral changes (periosteal appositions and remodeling) are observed on the anterior part of 12 vertebral bodies (8 thoracic and 4 lumbar vertebrae) as well as periosteal reaction (symmetrical, woven bone, *appliqué*) on several long bones, *i.e*. humerus, radius and ulnas (fig. 4). Vertebra and rib sample of this individual provided a positive aDNA specific sequence and clear peaks of lipid biomarkers. These data confirm tuberculosis in DJ II phase (Baker *et al*. 2015) (table 3).

– **i675-Dj individual** (DJ II), a child aged 8-10 years at

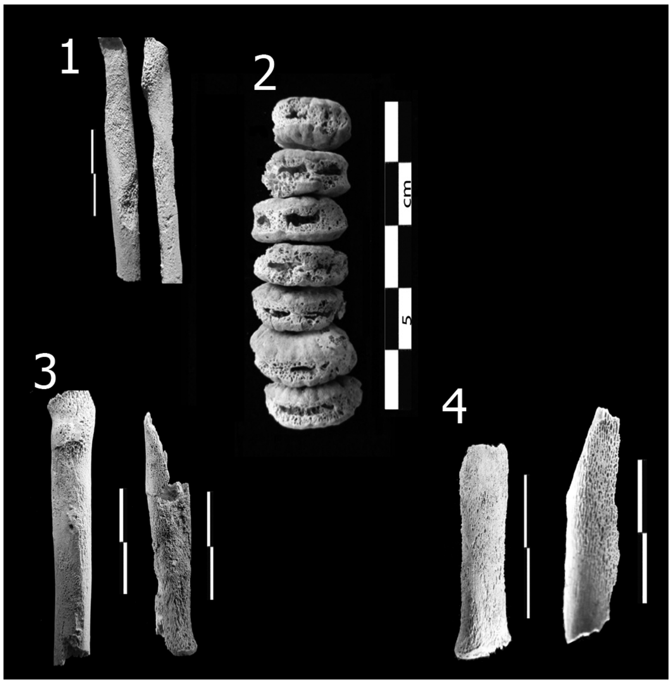


Fig. 4 – *Case i483-Dj from Dja'de el-Mughara (*ca *1 year old at death).* ***1****) Periosteal reaction on humerus (right and left);* ***2****) superficial vertebral changes;* ***3****) right radius and ulna;* ***4****) left radius and ulna (photo O. Baker).*

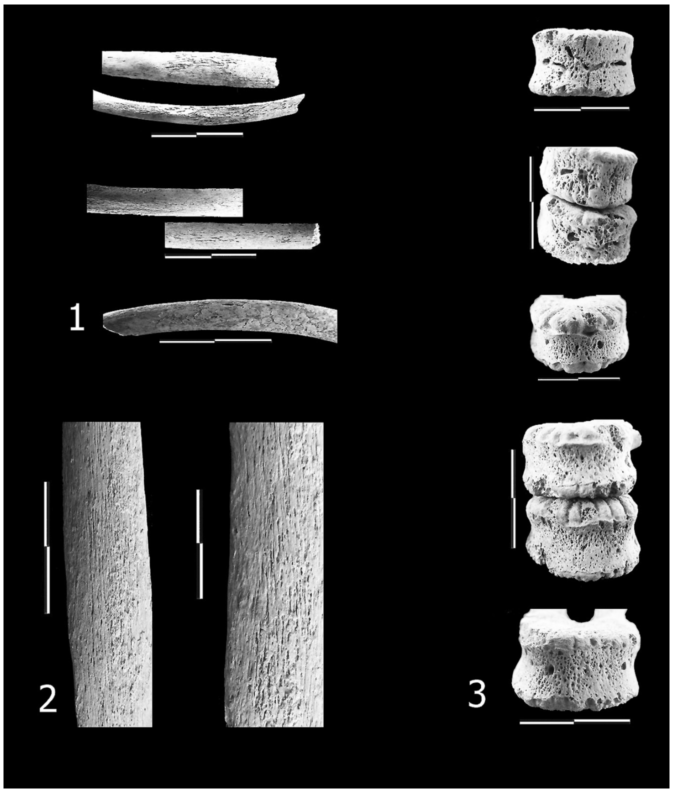


Fig. 5 – *Dja'de el-Mughara. Skeletal remains of a 8-10 year-old young child (case i675-Dj).* ***1****) Rib periostitis;* ***2****) periosteal reaction on long bones;* ***3****) superficial vertebral changes (photo O. Baker).*

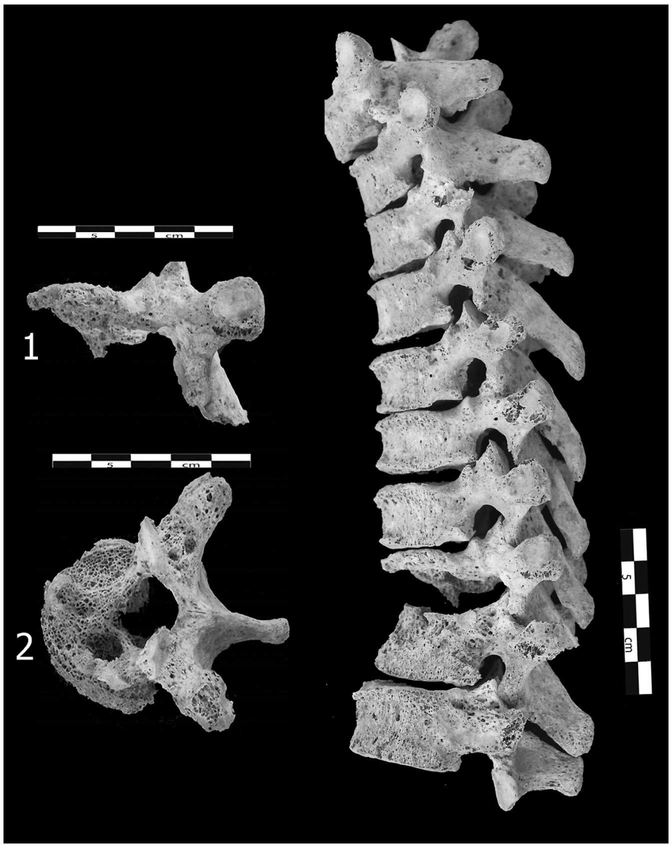


Fig. 6 – *Typical case of cystic spinal TB from Dja'de el-Mughara.* ***1****-****2****) 9th and 10th thoracic vertebrae of individual a304-Dj (photo O. Baker).*

death. The three associated lesions observed are: periosteal remodeling on the visceral surface of three ribs, hypervascularization on the anterior aspect of eight consecutive thoracic and three lumbar vertebrae, and periosteal reaction on both ulnas and tibias (Baker *et al*. 2015) (table 3; fig. 5).

– **a304-Dj individual** (DJ III), adult—sex determination is not possible—: macromorphological analysis and 3D reconstruction of laser surface acquisition revealed a typical aspect of spinal TB on the 9th and 10th thoracic vertebrae, as a cystic shape. The inferior part of the 9th thoracic vertebra is completely destroyed, and the upper part of the 10th thoracic vertebra shows cystic rounded cavitations expanded to the vertebral body, showing a space-occupying-mass aspect (Baker *et al*. 2015) (fig. 6).

– **B108-Dj individual** (DJ III), a child 4-5 years old at death: hypervascularisation of the anterior aspect of 9 vertebral bodies (6 thoracic and 3 lumbar vertebrae); early-stage of infectious spondylitis visible on 3D reconstruction from μCT scanning (Baker *et al*. 2015; Coqueugniot *et al*. 2015) (table 3; fig. 7).

Fig. 7 – *Case B108-Dj from Dja'de el-Mughara (4-5 years old at death); superficial vertebral changes (periosteal appositions and remodeling) (photo O. Baker).*

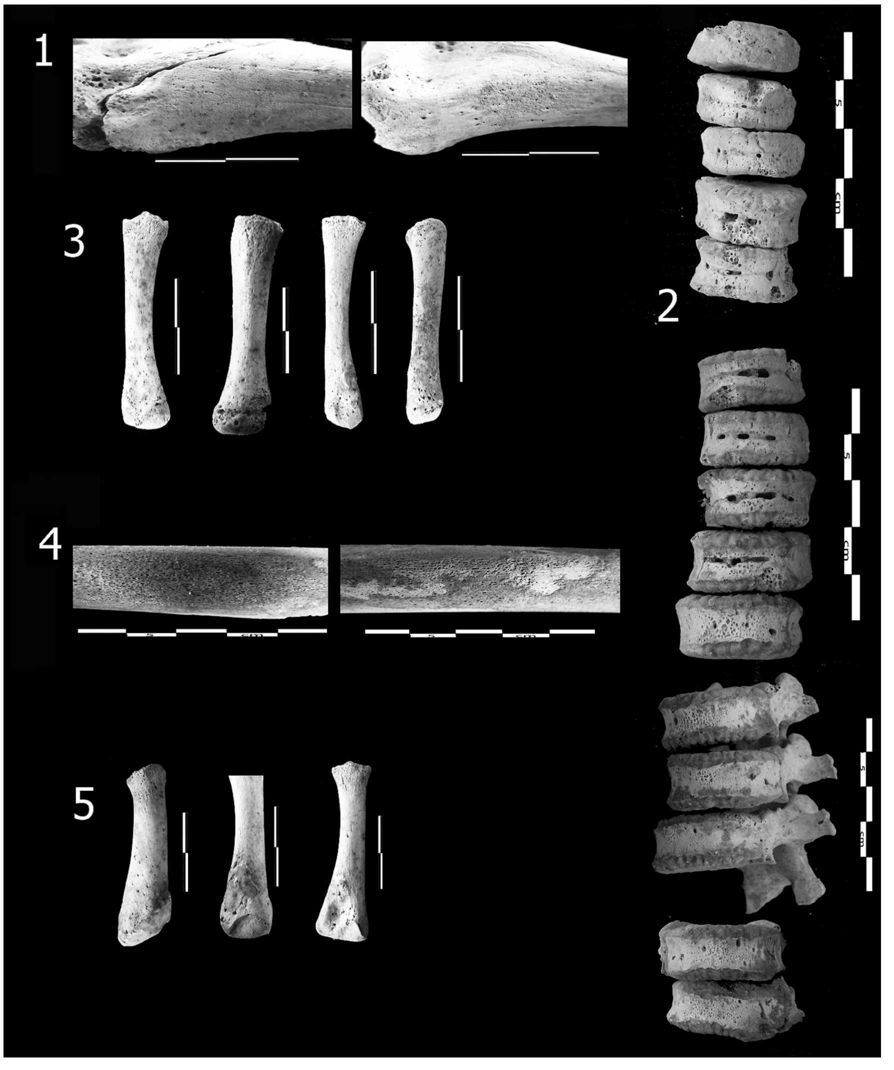


Fig. 8 – *Case i278-Dj from Dja'de el-Mughara (4-5 years old at death).* ***1****) Periosteal reaction on humerus (right and left);* ***2****) superficial vertebral changes;* ***3****) periosteal reaction four right metacarpal bone;* ***4****) periosteal reaction on right and left tibia;* ***5****) periosteal reaction on three left metatarsal (photo O. Baker).*

– **i278-Dj individual** (DJ II phase), a child of *ca* 4-5 years old at death: lesions are represented by symmetrical periosteal reaction on long bones (humerus and tibia) and on short tubular bones (6 metacarpals and 3 metatarsals); this aspect matches with criteria of hypertrophic osteoarthropathy. A periosteal reaction is also visible on the anterior part of the vertebral bodies of 7 thoracic and 4 lumbar vertebrae, besides an enlargement of the vascular foramina (anterior venous plexuses) (fig. 8).

– **C108-Dj individual** (DJ III phase), a child *ca* 2-3 years old at death: hypervascularisation (periostal appositions and remodeling) were observed on the anterior part of 11 vertebral bodies (8 thoracic and 3 lumbar vertebrae) (fig. 9).

Four other individuals from Dja'de el-Mughara (DJ III phase) present the same pathological pattern described previously on their anterior part of vertebral bodies (enlargement of the venous foramina, periosteal reaction) (figs. 4-5 and 7-9). These individuals are labeled I108-Dj (6-13 years old), R108-Dj (4-5 years), J108-Dj (*ca* 1 year), and i259-Dj (12-18 years) and were not described in our previous study (Baker *et al*. 2015).

Fig. 9 – *Case C108-Dj from Dja'de el-Mughara (2-3 years old at death); superficial vertebral changes (periosteal appositions and remodeling) (photo O. Baker).*

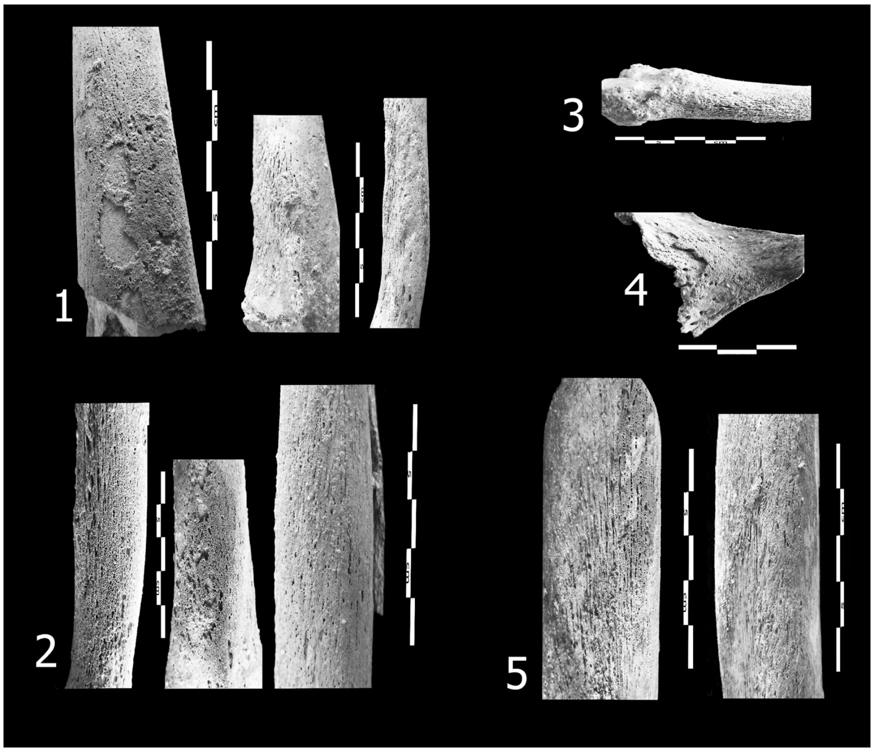


Fig. 10 – *Individual a509-Aw from Tell Aswad. Signs of HOA lesion observed on long bones.* ***1****) Right humerus, radius and ulna;* ***2****) left humerus, radius and ulna;* ***3****) left third metatarsal bone;* ***4****) fragment of left coxal;* ***5****) right and left tibias (photo O. Baker).*

TELL ASWAD

– **a509-Aw individual** (*Aswad récent*), a young adult *ca* 20-22 years old at death: periosteal lesion typical of hypertrophic osteoarthropathy, characterized by symmetrical diffuse periosteal reaction, uni and multi-lamellar with an *appliqué* aspect, involving both long and short bones (fig. 10). Using µCT, changes of early process of osteomyelitis are observed on the distal part of the right ulna (early stage of spina ventosa). Mycocerosate and mycolipenate profiles of MTBC infection were recorded on fibula samples (Baker *et al*. 2015) (table 3).

DISCUSSION AND CONCLUSION

Both sites, Dja'de el-Mughara and Tell Aswad, Syria, provided new and clear paleopathological evidence of human tuberculosis, demonstrated by pluridisciplinary methods (Baker *et al*. 2015; Coqueugniot *et al*. 2015). This paleopathological study was made possible thanks to the recent development of anthropological studies on Neolithic human remains discovered in Syria, particularly in the framework of three PhD researches (Baker 2014; Chamel 2014; Khawam 2014).

PREVALENCE OF TB CHANGES

A rough prevalence of 6.5% (11/170) of TB paleopathological cases was obtained in the present study, corresponding to the percentage of individuals presenting skeletal changes attributed to TB. Indeed, we did not carried out at this stage any molecular investigations on visually 'healthy' skeletons (skeletons that do not exhibit pathological changes evoking TB), that could increase this frequency. Therefore, when one considers that the mean percentage of skeletal involvement among the individuals 'infected' by TB (that differs from the percentage of individuals 'exposed' to TB) is commonly ranging from 1 to 3% according to modern data (Khalil 2008), then it can be said that this Neolithic population was exposed to a very high TB infection risk.

For Dja'de el-Mughara, we have identified ten individuals having skeletal changes evoking TB infection, all belonging to the Early PPNB period (DJ II and III) with a crude prevalence of 8.6% (10/116) (table 4); the prevalence observed for the three cases from DJ II is relatively higher than the seven cases identified from DJ III (15.0% *vs* 7.3% respectively; table 4), but this difference is not statistically significant at a level of significance of 0.05 (z=1.117). However, the high proportion of immature individuals among the total number of affected individuals is noteworthy here. Indeed, only one adult is affected in DJ III (crude prevalence = 7.3%; table 4) while all the other cases are from immature individuals.

For Tell Aswad, only one adult individual having TB changes has been identified, the crude prevalence is therefore estimated to 3.6% (1/40) (table 5). But we must keep in mind that we have not had the opportunity to examine the immatures from this site, which represent an important part (almost half of the collection).

Typical lesions are observed on one adult (crude prevalence = 2.7%, corrected prevalence = 8.3%) corresponding to a healed stage and on one immature (crude prevalence = 1.7%, corrected prevalence = 11.1%) corresponding to an active stage (table 4). Hypertrophic osteoarthropathy (active stage) was present on three individuals from Dja'de el-Mughara (corrected prevalence = 50.0%) and one individual from Tell Aswad (corrected prevalence = 3.6%), spina ventosa (active) on one individual (corrected prevalence = 4.8%), rib lesions (active) on one case (crude prevalence = 16.7%, corrected prevalence = 25.0%), and vertebral hypervascularization (active) on nine immature cases—among them 3 individuals from DJ II (crude prevalence = 50.0%, corrected prevalence = 37.5%) and 6 individuals from DJ III (crude prevalence = 10.2%, corrected prevalence = 65.6%; table 4).

The lesions observed on the immature individuals were only active ones, therefore we can assess that the majority of the immature individuals of this sample died most probably "of" tuberculosis, rather than "with" tuberculosis. Then, we can say that the death toll due to tuberculosis among the youngest part of the population of Dja'de el-Mughara was significant, maybe because of a special virulence of the TB strain during the childhood in this PPNB population.

SPECIFIC TREATMENT FOR THE AFFECTED INDIVIDUALS?

The funerary practices at Dja'de el-Mughara are various (Chamel 2014) : 13 deposits were found in the DJ II phase, including primary burials, scattered bones, foundation deposits and plural burials, primary and secondary. The three individuals with traces of tuberculosis from this phase have been buried in primary and single burials, two of them inside a building (i483-Dj and i278-Dj), and one outside (i675-Dj). The i483-Dj deposit seems to be a foundation deposit, inside a rectangular building along the internal wall, lying on his/her side in a flexed position. The i278-Dj deposit was found inside a rectangular house, in a corner, in a seated position. Grave i675-Dj has no link with any structure of the site, but was also

Table 4 – *Dja'de el-Mughara and Tell Aswad. Crude and corrected prevalence of TB on the bone sample by age and type of lesion.*

Site	Type of lesion	Immature			Adults			Total	
		Crude prevalence		Corrected prevalence (%)	Crude prevalence		Corrected prevalence (%)	Crude prevalence	
		n/N2	%		*n/N2*	%		*n/N2*	%
Dja'de el-Mughara DJ II	HOA	3/6	*50.0*	*50.0*	0/14			3/20	*15.0*
	PR	1/6	*16.7*	*25.0*	014				
	SAVChs	3/6	*50.0*	*37.5*	0/14				
Dja'de el-Mughara DJ III	CVT				1/37	*2.7*	*8.3*	7/96	*7.3*
	ESS	1/59	*1.7*	*11.1*					
	SAVChs	6/59	*10.2*	*65.6*					
Total DJ II and DJ III	-							10/116	*8.6*
"Aswad récent"	HOA				1/28	*3.6*	*4.0*	1/28	*3.6*
	OM				1/28	*3.6*	*4.8*		

Paleopathological changes: HOA: hypertrophic osteoarthropathy; PR: periostitis of rib; SAVCh: superficial anterior vertebral changes; CVT: cystic vertebral tuberculosis; ESS: early stage of spondylitis; OM: osteomyelitis.

Table 5 – *Dja'de el-Mughara and Tell Aswad Crude prevalence of TB on the bone sample.*

Site	Number of cases	Immature		Adults		Total	
		n/N1	%	*n/N1*	%	*n/N1*	%
Dja'de el-Mughara DJ II	3	3/8	*37.5*	0/18	–	3/26	*11.5*
Dja'de el-Mughara DJ III	7	6/60	*10.0*	1/39	*2.6*	7/99	*7.1*
Total Dja'de el-Mughara, with 130 individ.	10	9/69	*13.0*	1/61	*1.6*	10/130	*9.0*
"Aswad récent"		–	–	1/40	*2.5*	–	–

found in a seated position. It is noticeable that the i675-Dj and i278-Dj burials were the only ones with the body buried in a seated position for the DJ II phase but also for the whole site.

For the DJ III phase, 16 funerary deposits were discovered, six primary and single burials, eight plural burials (collective or simultaneous, primary and secondary), and two secondary burials, possibly foundation deposits.

The individual from grave a304-Dj was part of a plural burial, not entirely excavated, without any link with architecture, while all the other skeletons with traces of tuberculosis were found in relation with a peculiar building, named the "House of the Dead" due to its mortuary function. This building gathered 80 skeletons in 11 funerary deposits, both primary and secondary; no selection according to the age at death or sex was pointed out (Chamel 2014; Chamel and Coqueugniot in press).

Grave i259-Dj was discovered outside this building, in a flexed position. The other individuals from DJ III phase infected with tuberculosis were discovered inside the "House of the Dead" (Chamel 2014), and they were all part of the last burial of the building, grave 108. This burial yielded the remains of 16 individuals, with an age-at-death ratio of seven immatures for one adult, maybe indicating a selection. Two phases were separated with sediment, possibly indicating a temporary closure of the grave. The first phase gathered primary burials, mostly disturbed, while in the second phase the primary deposits were not disturbed. We can notice simultaneous deposits of several individuals, two by two or three by three, with a possible will of staging (fig. 2B).

R108-Dj is part of the first phase of the burial, while the others are part of the second phase (B108-Dj, C108-Dj, I108-Dj and J108-Dj). C108-Dj and I108-Dj were buried together, C108-Dj across the legs of I108-Dj (Chamel 2014). B108-Dj is the last individual buried in the House of the Dead (fig. 2B).

The presence of five individuals with tuberculosis in one burial (108), a frequency of more than 30%, could indicate a gathering of the individuals with the same infection in this grave.

At Tell Aswad, the young adult named "a509-Aw" (a.k.a. 509-H1 according to the excavators) has been inhumed in the mixed collective burial (primary and secondary) 509, located in funeral area I (level B0, Late PPNB), and containing at least three subjects (MNI) (Khawam 2014). This young acephalous adult was buried in a flexed position, placed on his/her left side, on a flat basket of spiral point, coated with clay (D. Stordeur, personal comm.), a cowry shell deposited on the left foot. A 5-9 year-old immature individual (509-H2) consisting only of a cranium was placed on the back of 509-H1 and outside the flat basket. The third individual, 509-H3 (of undetermined age and sex) is only represented by the lower limbs placed parallel to and overlapping 509-H1 at the knee height.

A funeral deposit comprising three articulated gazelle vertebrae, a tortoise shell and a broken arrowhead was found in connection with a fireplace juxtaposed to the burial 509 from the west side. These elements, likely of high symbolic value, could correspond to dishes and / or offerings (Khawam 2014; Khawam *et al*. 2016).

The funerary treatment applied to the 509-H1 subject, infected with active pulmonary tuberculosis which probably caused his/her death, follows specific rules. The burial was placed on the margin of the area. The funeral practices were carried out at several times. The interior design of the burial place was subject to special care. Finally, a funeral furnace, rather rare and precious in the funeral area, accompanied this dead individual (Khawam 2014). The precise and detailed documentation raises inevitably the question of a relationship between the pathological cause of death and the very special funeral treatment of the dead. However, only the examination of all the human remains from Tell Aswad for the diagnosis of tuberculosis at the level of the whole population will allow to confirm the existence of such a link. Nevertheless, at least at the level of one burial context, the link between the disease and the funerary practice can be evoked (Khawam *et al*. 2016).

IMPLICATIONS OF THE ANTIQUITY OF HUMAN TUBERCULOSIS IN THE LEVANT

Among the identified cases of TB infection, three of them are observed at Dja'de el-Mughara (DJ II phase, 8800-8500 cal. BC) where the domestication of the aurochs has not yet been reported. Other cases are dated from the end of Early PPNB and the Middle PPNB that correspond to the early stages of animal domestication (Helmer *et al*. 2005; Peters *et al*. 2005). At Dja'de el-Mughara biometric analysis of cattle bones dated to the most recent Early PPNB occupations (DJ III phase) showed a significant reduction of sexual dimorphism compared to the PPNA populations (Helmer *et al*. 2005). These changes in size at a population level were interpreted as resulting from a human selection and herd management. At the same period, evidence of goat, sheep and pig domestication were found at the Early PPNB site of Nevalı Çori, about 100 km far north in the Upper Euphrates Valley (Peters *et al*. 1999 and 2005). In addition, we know that cattle were introduced on the island of Cyprus at approximately 8400-8300 cal. BC (Shillourokambos phase 1A; Vigne 2013). Therefore, given the current state of zooarchaeological research for the Northern Levant, we cannot exclude that some kind of cultural control over part of *Bos* population could have been already practiced during DJ II phase, as an ongoing process, although only further data will resolve this issue.

The present study confirmed that human tuberculosis was present in the Near East as early as about 10,500 years BP. Dja'de el-Mughara and Tell Aswad sites represent, up to now, the earliest known paleopathological evidence of human tuberculosis.

This occurrence of human tuberculosis predating or possibly accompanying the beginnings of animal husbandry could support the model of human ancestral origin of the MTB complex (Brosch *et al*. 2002; Gutierrez *et al*. 2005) and is much more less in favor of the model of a recent emergence of MTBC *ca* 6 kyrs BP (Bos *et al*. 2014). More detailed paleopathological investigations on animal remains, including bone microstructure and molecular analyses, are presently underway and would provide a more complete picture of the tuberculous infection during the phase of emergence of domestication. Indeed, case of tuberculosis for Paleolithic times, molecularly confirmed, was reported on animal bones only (Rothschild *et al*. 2001). Therefore, pluridisciplinary analyses carried out on wild and domestic animals remains on these sites and on other Paleolithic and Early Neolithic sites in the World would help to precise the evolutionary link between human and animal tuberculosis at a global scale. It would be also of interest to reevaluate hypotheses formulated from Paleolithic human remains such as Kocabaş fossil from Turkey (Kappelman *et al*. 2008).

Therefore, two factors favoring the emergence and spread of modern human TB should be considered: a bacterial species jump from animal to human due to proximity with animal species during the first phases of domestication on one hand and sedentism, leading to a dramatic increase of the Neolithic human population density on the other one.

Our data do not exclude that the tubercle bacilli were already circulating in Paleolithic populations and spread with sedentism. Actually, archaeological data for the Near East support this model. The process of settling down in this region finds its roots in the Natufian culture at the end of the Pleistocene (Belfer-Cohen and Bar-Yosef 2000). Later, in the Middle Euphrates Valley for instance, several PPNA sites—*e.g*., Mureybet, Jerf el-Ahmar, Dja'de el-Mughara, Tell 'Abr—are fully sedentary settlements where subsistence economy was supplied by big and small game hunting (Gourichon 2004) and pre-domestic cultivation of cereals and pulses (Willcox 2013). While the mid-9th millennium BC constitutes a key period for understanding the remarkable coincidence between the earliest manifestations of human TB infections and the advent of animal domestication, this Neolithic period remains one of the most poorly documented archaeologically…

FURTHER WEST: TUBERCULOSIS AND THE NEOLITHISATION OF EUROPE

The antiquity of human tuberculosis in the Levant, considered as the cradle of Neolithization process, raises the question of the tempo of its spread into Europe. In this perspective, research on Neolithic cases in Central Europe should be carefully analyzed. Neolithic sites in Hungary such as Hódmezővásárhely-Gorzsa (Masson *et al*. 2015) or Alsónyék-Bátaszék (Pósa *et al*. 2015) and dating both from the first half of the 5th millennium BC are among the earliest European evidence of human tuberculosis, 2-3 millennia after our observations and could illustrate the pathway of tuberculous infection from the Levant to Western Europe.

The 7000-year-old skeletal remains of Hódmezővásárhely-Gorzsa are among the oldest molecularly confirmed ancient TB cases in Europe (Masson *et al*. 2013 and 2015; Pálfi *et al*. 2015). Alsónyék-Bátaszék site revealed a typical case of Pott's disease (Köhler *et al*. 2014), confirmed by the presence of MTBC DNA, detected as well in three other individuals of the same grave group (Pósa *et al*. 2015). These morphological and molecular evidence confirm the occurrence of TB in Neolithic populations of Europe at least seven thousand years ago.

Regarding the diffusion of human tuberculosis, demographic booming and human prehistoric migrations (Szécsényi-Nagy *et al*. 2015) as a consequence of Neolithic new way of life, firstly marked by sedentism, may have played a greater role than only domestication. Whichever scenario we adopt, the first or the second one, the role of the Levant seems crucial as the primary scene of events in the history of human tuberculosis.

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