Tuberculosis 95 (2015) S4–S12

Contents lists available at ScienceDirect

Tuberculosis

journal homepage: http://intl.elsevierhealth.com/journals/tube

Human tuberculosis predates domestication in ancient Syria

Oussama Baker [a], Oona Y.-C. Lee [b], Houdini H.T. Wu [b], Gurdyal S. Besra [b], David E. Minnikin [b], Gareth Llewellyn [c], Christopher M. Williams [c], Frank Maixner [d], Niall O'Sullivan [d], Albert Zink [d], Bérénice Chamel [e], Rima Khawam [e], Eric Coqueugniot [e], Daniel Helmer [e], Françoise Le Mort [e], Pascale Perrin [f], Lionel Gourichon [g], Bruno Dutailly [h], György Pálfi [i], Hélène Coqueugniot [h, j], Olivier Dutour [a, h, k, *]

[a] Laboratoire d'Anthropologie biologique Paul Broca, Ecole Pratique des Hautes Etudes, Paris, France
[b] Institute of Microbiology and Infection, School of Biosciences, University of Birmingham, B15 2TT Birmingham, UK
[c] National Mass Spectrometry Service Centre, School of Medicine, Grove Building, Swansea University, Swansea, UK
[d] Institute for Mummies and the Iceman, European Academy, Viale Druso, 1, 39100 Bolzano, Italy
[e] Laboratoire Archéorient (UMR 5133, CNRS, Université de Lyon 2), Maison de l'Orient et de la Méditerranée – Jean Pouilloux, 7 rue Raulin, 69365 Lyon Cedex 7, France
[f] Université Montpellier 2 – MIVEGEC IRD/UR 224 – CNRS/UMR 5290, MIVEGEC – Centre IRD de Montpellier, 911 Avenue Agropolis, BP 64501, 34394 Montpellier Cedex 5, France
[g] Cultures et Environnements Préhistoire, Antiquité, Moyen Âge (CEPAM, UMR 7264, CNRS Université Nice Sophia Antipolis), Pôle Universitaire Saint-Jean-d'Angély (SJA3), 24 Avenue des Diables Bleus, 06357 Nice Cedex 4, France
[h] De la Préhistoire à l'Actuel: Culture, Environnement, Anthropologie (PACEA, UMR 5199, CNRS, Université de Bordeaux, Ministère de la Culture et de la Communication), Allée Geoffroy St Hilaire, CS 50023, 33615 Pessac Cedex, France
[i] Department of Biological Anthropology, University of Szeged, Dugonics Ter, Szeged, Hungary
[j] Department of Human Evolution, Max Planck Institute for Evolutionary Anthropology, Deutscher Platz 6, 04103 Leipzig, Germany
[k] Department of Anthropology, University of Western Ontario, London, Canada

Keywords:
Paleopathology of TB
Early neolithic
PPNB
Agriculture cradle
Domestication
Lipid biomarkers
Ancient DNA

SUMMARY

The question of pre-neolithic tuberculosis is still open in paleopathological perspective. One of the major interests is to explore what type of infection could have existed around the early stage of animal domestication. Paleopathological lesions evoking skeletal TB were observed on five human skeletons coming from two PPNB sites in Syria, which belongs to the geographical cradle of agriculture. These sites represent respectively pre-domestication phase (Dja'de el Mughara, Northern Syria, 8800-8300 BCE cal.) and early domestication phase (Tell Aswad, Southern Syria, 8200-7600 BCE cal.). MicroCT scan analyses were performed on two specimens (one per site) and revealed microscopic changes in favor of TB infection. Detection of lipid biomarkers is positive for two specimens (one per site). Initial molecular analysis further indicates the presence of TB in one individual from Dja'de. Interestingly, no morphological evidence of TB was observed on animal remains of wild and newly domesticated species, discovered in these sites. These observations strongly suggest the presence of human tuberculosis before domestication and at its early stages.

1. Introduction

Human tuberculosis is a specific disease caused by infection by a member of *Mycobacterium tuberculosis* complex. Although the previous widely accepted hypothesis that the human pathogen (*M. tuberculosis*) derived from cattle pathogen (*Mycobacterium bovis*) by contamination during the Neolithic, the new evolutionary scenario assess that *M. tuberculosis* actually represents a direct descendant of a most ancient strain that existed before the split of *Mycobacterium africanum* – *M. bovis* lineages from the *M. tuberculosis* lineage [1]. This suggests that the tubercle bacilli could have been already a human pathogen before animal domestication.

* Corresponding author. Laboratoire d'Anthropologie biologique Paul Broca, Ecole Pratique des Hautes Etudes, UMR PACEA, CNRS, Université de Bordeaux, Allée Geoffroy St Hilaire, CS 50023, 33615 Pessac Cedex, France. Tel.: +33 540008931; fax: +33 540002545.
E-mail address: olivier.dutour@ephe.sorbonne.fr (O. Dutour).

http://dx.doi.org/10.1016/j.tube.2015.02.001
1472-9792/

Paleopathological evidence of tuberculosis before the Neolithic is poorly documented. Lesions observed on extinct bison remains discovered in a natural trap cave in Wyoming and dated from 17000 BP, led to biomolecular identification of *M. tuberculosis* ancient DNA and lipid biomarkers [2]. A diagnosis of leptomeningitis tuberculosa was mentioned about endocranial lesions observed on a fossil hominin attributed to *Homo erectus* from Turkey [3], but this hypothesis was questioned by other authors [4]. As for human tuberculosis, most ancient cases are dated from 7000 yrs BCE in the Eastern Mediterranean. In the site of Atlit Yam, remains of an adult female and an immature individual presented paleopathological evidence of TB, confirmed by lipid biomarkers and aDNA analyses [5]. In the same geographic area, paleopathological evidences of tuberculosis were previously mentioned for contemporaneous site (Ain Ghazal, ca. 7250 BCE) [6]. These cases correspond to the development of the first farming and herding societies in the Fertile Crescent, known to be the cradle of agriculture.

In order to explore the link between human and cattle tuberculosis at the beginning of domestication process, the first purpose of this study is to investigate the paleopathological evidence of tuberculosis from two ancient Neolithic sites representing pre and early domestication phases in ancient Syria. This study will allow shedding further light on the debate concerning the origins and the evolutionary pathway of the *M. tuberculosis* complex, for example by dating the emergence of the modern strain of *M. tuberculosis.*

2. Materials and methods

2.1. Archaeological material

Studied material is coming from two Pre-Pottery Neolithic B (PPNB) sites from Syria, Dja'de el Mughara and Tell Aswad.

The pre-domestic Neolithic site of Dja'de el Mughara is located on the left bank of the Euphrates River, about 115 km N/W from Aleppo. It has been excavated from 1991 to 2010 in the framework of the French Archaeological Research Program in Syria, headed by Eric Coqueugniot [7]. The chronology of the human occupation ranged from 9310 to 8200 BCE cal. It has been divided in 3 chrono-cultural periods. The first one (DJ 1) corresponds to the end of PPNA, transition phase to early PPNB (9310-8830 BCE cal.). Human remains associated to this period are scarce: only five individuals were discovered, represented by four adult skulls and a fragmentary skeleton of an adolescent. The second period (DJ 2) corresponds to early PPNB and is dated from 8800 to 8500 BCE cal. Human settlement is represented by 13 burials that provided skeletal remains of 26 individuals (8 immature individuals and 18 adults). The third period (DJ 3), corresponding to the end of early PPNB, is dated from 8540 to 8290 BCE cal. Human remains associated to this period are more numerous: 99 individuals were identified (37 adult and 62 immature individuals). More than 70 are coming from collective burials (Houses of the Dead). After 8200 BCE, the site was abandoned till the 7th millennium.

During this period, people still rely on hunting and gathering. Indeed, the wild resources were still dominating the diet, and there is no morphological evidence that either cereals or animals were domesticated. At the “DJ 3” phase, however, some clues of pre-domestication could be recognized, both for plants (wild cereals) and animals (aurochs) [7,8].

For this site, skeletal remains of 130 individuals were examined by two researchers of this sudy (OB and BC).

The Neolithic site of Tell Aswad located around 35 Km from East/Southeast Damascus (Southern Syria) has been discovered in 1967 by H. de Contenson, and excavations were performed between 2001 and 2006 by a French-Syrian archeological team co-headed by Danielle Stordeur and Bassam Jammous [9].

Numerous skeletal remains were discovered, representing 119 individuals, dating from middle and end of PPNB (8200-7600 BCE cal). The domestic resources have been strongly used since the middle PPNB: cereals are derived from an agricultural economy, and exploitation of domestic animals increases in this period, while hunting decreases.

2.2. Methods

2.2.1. Anthropology and paleopathology

All the skeletal material was carefully examined in the storage place on site, both for anthropological and paleopathological purposes. Possible changes due to TB were systematically researched on spine, bones and joints. Diagnostic was based upon the morphology and distribution of the lesions according to criteria commonly accepted in clinical and paleopathological literature [10–13]; including aspects of spondylodiscitis, osteoarthritis and periostitis. Age estimation and sex determination of the immature and adult individuals were performed according to a set of different methods currently used in biological anthropology [14–16].

The skeletal pieces matching with these criteria were selected and analyzed by different methods. Morphological methods have used digital imaging acquisitions by laser and X-ray (μCT) with volumetric reconstructions. Lipidic biomarkers were detected by HPLC [17] and MTBC specific sequence motifs were targeted by ancient DNA based analysis.

In the same time, animal bones found in these sites (wild and domesticated species) were studied by two researchers of this study (DH and LG). No paleopathological lesion was observed. A sample of 9 cattle bones from Dja'de el Mughara was selected for further analyses (lipid biomarkers and ancient DNA).

2.2.2. Digital imaging

Lesions present on 2 thoracic vertebrae of individual Dja'de 304 were digitalized by 3D laser scan at low energy (class II), without contact at a mean resolution lower than 0.5 mm. Contrarily to X-rays, this surface laser acquisition allows saving morphological information before sampling for molecular analysis while protecting ancient DNA from ionization. Moreover, it allows the 3D printing of the lesions by rapid prototyping (Eden250™ 3D Printing System, Objet, with horizontal layers of 16 μm) using VIRCOPAL® technology [18].

Two specimens were analyzed by microtomodensitometry: distal ulna of individual Aswad 509 on a GE Healthcare eXplore Locus microCT at a resolution of 20 μm and lumbar vertebra of young immature individual Dja'de B108 on a GE V/tome/x at 3 μm.

3D reconstructions were performed using TIVMI® software program [19].

2.2.3. Lipid extraction

Analyses were performed on 2 specimens presenting paleopathological lesions (Dja'de sp483 and Tell Aswad 509). The Dja'de skeleton sp483 provided 14 vertebrae (Laboratory Numbers DV1 to DV14) and 36 rib (Laboratory Numbers DR1 to DR36) samples for study. Eleven fibula fragments were available from Tell Aswad skeleton 509 (Laboratory Numbers AF1 to AF11). Bone samples AF1 (548 mg), AF3 (573 mg), AF11 (473 mg), DR13 (256 mg), DR36 (316 mg), DV7 (214 mg) and DV13 (70 mg) were chosen for lipid biomarker analysis. Specimens were hydrolysed by heating with 30% potassium hydroxide in methanol (2 ml) and toluene (1 ml) at 100 °C overnight [20]. In parallel, standard biomass from *M. tuberculosis* was processed. Long-chain compounds were extracted as described previously [20] and the extract was treated with