Positive Diagnosis of Ancient Leprosy and Tuberculosis using Ancient DNA and Lipid Biomarkers

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Table S1. Summary of ancient tuberculosis methodology and biomarker findings according to date of publication.

Authors	Year	Region	Period	Techniques used
Spigelman & Lemma [4]	1993	Scotland. Turkey. Borneo.	17th cent. Byzantine. Pre-contact.	Skeletal remains. Repetitive sequence IS*6110*. PCR amplicon 123bp.
Salo *et al*. [5]	1994	Chiribaya Alta, South Peru.	11th cent. Pre-Columbian.	Mummified tissue. Nested PCR IS*6110* 123/97bp. Cloned and sequenced amplicon.
Arrieza *et al.* [22]	1995	Arica, Chile.	Pre-Columbian.	Nested IS*6110* PCR 123/97bp. Confirmed by *Sal I* restriction enzyme digestion.
Baron *et al.* [23]	1996	Göttingen, Germany.	Late 19th cent. – 1918 CE.	PCR 123bp & *Sal I* digestion. MTB positives in bones without lesions.
Taylor *et al*. [41]	1996	Royal Mint, London, UK.	1350–1538 CE.	Nested IS*6110* PCR 123/92bp. Human DNA *Alu* repetitive element.
Nerlich *et al*. [42]	1997	Thebes- West, Upper Egypt.	1550–1080 BCE.	Pleural adhesions in mummy. Mycobacterial surface antigen target 133bp was sequenced.
Braun *et al*. [43]	1998	Canada & Mississippi.	15th cent. 11th cent.	PCR IS*6110* 123/92bp. Cloned and sequenced amplicons.
Donoghue *et al*. [9]	1998	Karkur, Israel.	600 CE.	PCR IS*6110* 123/92 bp. Amplicons sequenced. MTB mycolic acid lipid biomarkers.
Gernaey *et al.* [10]	1998	Newcastle upon Tyne, UK.	19th cent.	MTB mycolic acid lipid biomarkers.
Taylor *et al.* [44]	1999	Royal Mint, London, UK.	1350–1538 CE.	Hot-start PCR targeting S*6110*, *mtb40*, *oxyR*, *rpoB*. Line hybridization assay, spoligotyping, DNA sequencing.
Gernaey *et al.* [10]	2001	Addingham, Yorkshire, UK.	Early Mediaeval. Anglo-Saxon.	Nested PCR IS*6110* 181bp. MTB mycolic acid lipid biomarkers.
Rothschild *et al.* [45]	2001	Wyoming, USA.	17,000 BP. Natural Trap Cave bison.	PCR IS*6110* & ribosomal protein S12. Spoligotyping and principal components analysis indicate not *M. bovis*.
Zink *et al.* [24]	2001	Thebes-West, Upper Egypt.	3000 & 2120–500 BCE.	PCR IS*6110* 123bp. Confirmed by restriction enzyme digestion or directly sequenced.
Mays & Taylor [46]	2002	Wharram Percy, Yorkshire, UK.	10th–16th cent.	PCR IS*6110*, *oxyR* and sequenced. Showed Hypertrophic Osteoarthropathy (HOA) linked to TB.
Spigelman *et al.* [47]	2002	Scotland. Turkey. Borneo.	17th cent. Byzantine. Pre-contact.	PCR IS*6110* 123/92bp, INS-1/INS-2 246bp. Ribosomal protein S12-F/S12-R. Amplicons sequenced in 2 laboratories.
Fletcher *et al.* [25]	2003	Vác, Hungary.	18th cent.	350 samples from 168 bodies. IS*6110* positives screened for other target sites. Genotyped into groups 2 & 3.

Taylor *et al.* [48]	2003	Berlin, Germany.	1901 CE from 19th cent. Koch patient.	PCR IS*6110*, IS*1081*, *katG*, *gyrA*, *oxyR*, *pncA*, TbD1. Flanking & internal primers – all typical of MTB.
Zink *et al.* [49]	2003	Thebes-West, Upper Egypt.	2050-1650 BCE. ca. 500 BCE.	PCR IS*6110* 123bp. Spoligotyping – *M. tuberculosis.* Middle Kingdom – *M. africanum.*
Bathurst & Barta [26]	2004	Ontario, Canada.	16th cent. Iroquoian dog.	PCR canine aDNA. MTB IS*6110* 123/92bp.
Donoghue *et al.* [36]	2005	Egypt. Hungary. Israel. Sweden.	4th–5th cent. 10th–16th cent. 1st cent. 10th–13th cent.	PCR ML RLEP 129/99bp; MTB IS*6110* 123/92bp. Several specimens co-infected with ML and MTB.
Taylor *et al.* [50]	2005	Dorset, UK.	2200 BP. Iron Age.	PCR IS*6110*, IS*1081*, *katG*, *gyrA*, *oxyR*, *pncA*, TbD1. Flanking confirmed by sequencing.
Spigelman *et al.* [51]	2006	Vác, Hungary.	18th cent.	Demonstrated co-morbidity – TB and Langerhans cell histiocytosis.
Taylor *et al.* [27]	2007	Tuva, South Siberia.	Iron Age.	PCR IS*6110*, IS*1081*, TbD1, RD regions 4, 12, 13, 17, *oxyR*285 (A), *pncA*169 (G) indicate *M. bovis* amplicons sequenced.
Hershkovitz *et al.* [28]	2008	Atlit Yam, Israel.	9,000 BP. Sub-sea village.	PCR IS*6110*, IS*1081*, TbD1. MTB mycolic acid lipid biomarkers.
Matheson *et al.* [52]	2009	Shroud Cave, Jerusalem, Israel.	1st cent.	PCR - human haplotypes. MTB, ML co-infection. Family tomb - some related remains.
Murphy *et al.* [53]	2009	Tuva, South Siberia.	Iron Age.	Quantitative PCR used to estimate the amount of surviving *M. bovis* aDNA.
Redman *et al.* [54]	2009	Coimbra, Portugal.	1837–1936 CE. Documented TB.	MTB mycolipenic and mycocerosic acid lipid biomarkers.
Donoghue *et al.* [55]	2010	Thebes, Egypt.	600 BCE.	MTBC aDNA and mycolic acids in Granville mummy.
Donoghue *et al.* [56]	2011	Vác, Hungary.	18th cent.	Identified MTB aDNA in pulmonary and extra-pulmonary tissue. Localized and disseminated disease.
Minnikin *et al.* [12]	2011	Hungary. Turkey. Sweden. UK.	7th–15th cent. 8th–9th cent. 10th–13th cent. 19th cent.	MTB and ML mycolic acid lipid biomarkers in residues after DNA extraction.
Nicklisch *et al.* [57]	2012	Saxony-Anhalt, Germany.	5,400–4,800 BCE.	MTBC aDNA in long bones. TbD1 region intact.
Bouwman *et al.* [30]	2012	Leeds, UK.	19th cent.	Hybridization capture at specific regions of MTB genome. WGS by oligonucleotide ligation and detection.
Corthals *et al.* [58]	2012	Salta, Argentina.	500 BP.	PCR and proteomic profiles of Andean mummy tissue using 16srRNA, MTB40, MTB41, *hsp65* primers. Conclusions on host immune response.
Lee *et al.* [13]	2012	Kiskundorozsma-Daruhalom dűlő II, Szeged, Hungary.	7th cent.	Mycolic and mycocerosic acids confirm MTB/ML co-infection.
Lee *et al.* [29]	2012	Wyoming, USA.	17,000 BP. Natural Trap Cave bison.	MTBC mycolic (degraded), mycolipenic and mycocerosic acid and phthiocerol lipid biomarkers.
Masson *et al.* [59]	2013	Szeged, Hungary.	7,000 BP.	MTB aDNA IS*1081.* MTBC mycolic (degraded) mycolipenic and mycocerosic acid lipid biomarkers.
Chan *et al.* [31]	2013	Vác, Hungary.	18th cent.	Metagenomic analysis of MTB sequences related to modern strains in Germany. Mixed MTB infections – 2 strains.
Lairemruata *et al.* [60]	2013	Fayum, Lower Egypt.	30 BCE–300 CE.	Detected MTB and *Plasmodium falciparum* aDNA, cloned and sequenced. Identified single infections and 4 mixed infections.

Bos *et al*. [32]	2014	Southern Peru.	1028–1280 CE.	MTBC DNA capture assay for 5 target sites, sequenced on Illumina MySeq. *M. pinnepedii* infection.
Dabernet *et al.* [61]	2014	Yakutia, Eastern Siberia.	16th–19th cent.	Frozen mummies – used IS*6110* SNP typing by SNaPshot method. MTB strains PGG2-SCG-5 (European).
Müller *et al.* [62]	2014	UK and Europe	2nd–19th cent.	MTB aDNA in 10/34 samples - typed SNPs, large sequence polymorphisms, genotyped. 1 mixed infection.
Borowska-Strugińska *et al*. [63]	2014	Kujawy Region, Central Poland.	Neolithic.	MTB mycolic acid biomarkers by direct mass spectrometry.
Baker *et al*. [64]	2015	Dja'de el Mughara, Syria. Tell Aswad, Syria.	8800–8300 BCE. 8200–7600 BCE.	IS*6110* PCR, mtDNA, micro-CT scans. MTB mycolic (degraded), mycolipenic and mycocerosic acid lipid biomarkers.
Donoghue *et al.* [14]	2015	Austria. Hungary.	8th–9th cent. 7th–11th cent.	IS*6110* and *1081* PCR. 7 cases of MTB detected – 6 were co-infections with ML.
Hershkovitz *et al.* [65]	2015	Atlit Yam, Israel.	9,250–8,160 BP.	Bones of 64 humans and 5 cattle examined for MTB aDNA. No new positives.
Lee *et al*. [66]	2015	Atlit Yam, Israel.	9,000 BP. Sub-sea village.	MTB phthiocerol, mycolipenic and mycocerosic acid lipid biomarkers.
Kay *et al*. [33]	2015	Vác, Hungary.	18th cent.	WGS of 14 genomes. All were of lineage 4. 12 genotypes; some had multiple MTB strains.
Masson *et al*. [67]	2015	Szeged, Hungary.	7,000 BP.	MTB aDNA IS*6110* & *1081*. Mycolic and mycocerosic acid lipid biomarkers confirmed five MTB cases.

Numbered citations correspond to those in the main text. **Abbreviations: BCE**, Before Common Era; **bp**, base pairs; **BP**, Before Present, **CE**, Common Era; **ML**, *Mycobacterium leprae*; **MTB**, *Mycobacterium tuberculosis*; **MTBC**, *Mycobacterium tuberculosis* complex; **PCR**, Polymerase Chain Reaction; **SNP**, Single Nucleotide Polymorphism; **WGS**, Whole Genome Sequencing.

Table S2. Summary of ancient leprosy methodology and biomarker findings according to date of publication.

Authors	Year	Region	Period	Techniques used
Rafi *et al*. [6]	1994	River Jordan, Israel.	6th–7th cent.	PCR for 36kDa (530 bp) and 65kDa (439 bp) antigens.
Haas *et al*. [68]	2000	Rain/Lech, Germany.	15th–18th cent.	Nested RLEP PCR (372 /320 bp).
Taylor *et al*. [34]	2000	Newark Bay, Orkney, UK.	11th–12th cent.	Hemi-nested RLEP PCR 175/153 bp.
Donoghue *et al*. [35]	2001	Suraz, Poland. Hungary.	Mediaeval. 10th–11th & 15th cent.	Nested RLEP PCR (129/99 bp). Nested 18kDa antigen PCR (136/110 bp).
Spigelman & Donoghue [69]	2001	Jerusalem, Israel.	4th–7th cent.	As above.
Montiel *et al.* [70]	2003	Seville, Spain.	12th cent.	Nested RLEP PCR (149/97 bp).
Donoghue *et al*. [36]	2005	Akeldema, Himmon valley, Israel. The Dakhleh Oasis Kellis 2, Egypt. Püspökladány-Eperjesvölgy, Hungary. Björned, Sweden.	1st cent. 4th cent. 10th cent. 11th–13th cent.	Nested RLEP PCR 129/99 bp). Co-infection MTB complex. Nested IS6110 PCR (123/92 bp).
Taylor *et al*. [37]	2006	Blackfriars, Ipswich, UK. Wharram Percy, Yorkshire, UK.	13th–16th cent. 960–1100 CE.	Hemi-nested RLEP PCR(133/111 bp) and VNTR typing (99-131 bp).
Taylor *et al*. [11]	2009	Blackfriars, Ipswich, UK.	13th–16th cent.	RLEP PCR, VNTR, SNP genotyping.
		Devkesken 6, Uzbekistan.	1st–4th cent.	As above, plus ML mycolic acids.
Watson *et al*. [71]	2009	Radasinovci, Croatia. Odense, Denmark. Norwich, UK	8th–9th cent. 13th–16th cent. 10th–11th cent.	RLEP, PCR, SNP genotyping.
Matheson *et al.* [53]	2009	Akeldema, Himmon valley, Israel.	1st cent.	PCR for multiple *M. leprae, M. tuberculosis* and human (mtDNA, amelogenin, and sex chromosome loci).
Monot *et al.* [18]	2009	Dakhleh Oasis, Egypt. Ipswich, UK. Denmark. Turkey. Hungary. Hungary. Croatia.	4th–5th cent. 13th–16th cent. 13th–16th cent. 8th–9th cent. 7th cent. 10th–11th cent. 8th–9th cent.	SNP genotyping.
Suzuki *et al*. [72]	2010	Aomori, Japan.	8th–20th cent.	WGA-PCR plus SNP genotyping.
Taylor & Donoghue [20]	2011	Wharram Percy, Yorkshire, UK. Blackfriars, Ipswich, UK. Turkey. Prague, Czech Republic. Hungary. Hungary. Hungary.	10th–12th cent. 13th–16th cent. 8th–9th cent. 9th cent. 7th cent. 10th cent. 11th cent.	VNTR genotyping of 3 loci (99 -131 bp).
Minnikin *et al.* [12]	2011	Hungary. Turkey. Sweden. UK.	7th–15th cent. 8th–9th cent. 10th–13th cent. 19th cent.	MTB and ML mycolic acid lipid biomarkers in residues after DNA extraction.
Lee *et al.* [13]	2012	Kiskundorozsma-Daruhalom dűlő II, Szeged, Hungary.	7th cent.	Mycolic and mycocerosic acids confirm TB/ML co-infection.
Taylor *et al*. [38]	2013	Winchester, UK.	9th–13th cent.	Screening (RLEP / 18-kDa PCR). SNP genotyping. ML mycolic acids.
Schuenemann *et al.* [39]	2013	Winchester, UK. Sigtuna, Sweden. Refshale, Denmark. Jorgen, Denmark.	9th–13th cent. 10th–14th cent. 11th-12th cent. 13th–14th cent.	Whole Genome Sequencing (WGS). ML mycolic acids.
Economou *et al*. [73]	2013	Sigtuna, Sweden.	10th–14th cent.	RLEP PCR. SNP genotyping.

Mendum *et al.* [74]	2014	Winchester, UK.	955–1033 CE.	
		Winchester, UK.	1020–1162 CE.	WGS.
Donoghue *et al.* [14]	2015	Morrione, Italy.	6th–8th cent.	PCR for multiple *M. leprae*, and *M. tuberculosis* loci including nested RLEP PCR (129/99 bp) and nested 18kDa antigen PCR (136/110 bp). Co-infection of *M. leprae* and *M. tuberculosis* at several sites.
		Vicenne, Italy.	Mid–late 7th cent.	
		Kiskundorozsma-Daruhalom dűlő II, Szeged, Hungary.	7th cent.	
		Szentes-Kistőke, Hungary.	7th–8th cent.	
		Bélmegyer-Csömöki Domb, Hungary.	7th–9th cent.	
		Szarvas Grexa, Hungary.	Late 7th–9th cent.	
		Zwölfaxing, Austria,	8th–9th cent.	
		Kovuklukaya, Turkey	8th–9th cent.	
		Prušanky, Czech Republic	9th–10th cent.	
		Hajdúdorog-Gyúlás, Hungary.	10th cent.	
		Felgyő, Kettőshalmi-dűlő, Hungary.	11th cent.	
		Lászlófalva-Szentkirály, Hungary.	11th cent.	
Inskip *et al.* [40]	2015	Great Chesterfield, Essex, UK.	5th–6th cent.	PCR (RLEP & 18kDa), SNP & VNTR genotyping. ML mycolic and mycocerosic acids.
Molnár *et al.* [75]	2015	Bélmegyer-Csömöki Domb, Hungary.	7th–9th cent.	Mycolic, mycolipenic, mycocerosic acids MTB/ML co-infections.
Roffey *et al.* [76]	2017	Winchester, UK. Pilgrim.	1020–1162 cal. CE.	PCR (RLEP & 18kDa), SNP & VNTR genotyping.

Numbered citations correspond to those in the main text. **Abbreviations: bp**, base pairs; **CE**, Common Era; **ML**, *Mycobacterium leprae*; **MTB**, *Mycobacterium tuberculosis*; **PCR**, Polymerase Chain Reaction; **RLEP**, Repetitive sequence in the *Mycobacterium leprae* genome; **SNP**, Single Nucleotide Polymorphism; **VNTR**, Variable Number Tandem Repeats; **WGA**, Whole Genome Amplification; **WGS**, Whole Genome Sequencing.